FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research

Meeting of:

The Blood Products Advisory Committee

Open Session

May 13, 2015

FDA White Oak Conference Center
Building 31, Room 1503
10903 New Hampshire Ave.
Silver Spring, MD 20993

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PROCEEDINGS (8:30 a.m.)

Agenda Item: Opening Remarks

DR. JACKSON: I will now call to order the 112th meeting of the Blood Products Advisory Committee. Thank you very much for coming.

We will start with introductions and then Bryan Emery will read the conflict-of-interest statement.

I will start with myself. I'm Brooks Jackson, chair of the committee, dean of the School of Medicine and vice president for health sciences at the University of Minnesota, and a transfusion medicine specialist.

DR. BONILLA: Francisco Bonilla, clinical allergist/immunologist from Boston Children's Hospital.

DR. SIMON: Good morning. I'm Toby Simon, a senior medical director with CSL Behring, industry representative. A background in hematology and transfusion medicine.

DR. SCHEXNEIDER: Commander Katherine Schexneider, US Navy. I'm the medical director of transfusion services at Walter Reed, Bethesda. I have a clinical background in clinical transfusion medicine.

DR. SANDBERG: Good morning. I'm Professor Sandberg, professor of mathematics at Framingham State University.

DR. BAKER: Good morning. Judith Baker, a temporary consumer member today, public health director for the Center for Inherited Blood Disorders, and at UCLA in the Department of Pediatric Hematology/Oncology.

DR. NELSON: I'm Kenrad Nelson, professor of epidemiology at Johns Hopkins.

DR. MAGUIRE: James Maguire. I'm an infectious disease specialist at Brigham and Women's Hospital in Boston.

DR. DEMARIA: Al DeMaria. I'm the medical director, Bureau of Infectious Disease, Massachusetts Department of Public Health, and state epidemiologist for Massachusetts, also the president of the Council of State and Territorial Epidemiologists. I'm a temporary member today.

DR. STOWELL: I'm Chris Stowell. I'm the director of the Blood Transfusion Service at Mass General Hospital in Boston.

DR. RAGNI: Maura Ragni, professor of medicine at the University of Pittsburgh and director of the Hemophilia Center there.

DR. DURKALSKI: Valerie Durkalski, professor of biostatistics at the Medical University of South Carolina.

DR. BASAVARAJU: Sridhar Basavaraju, medical officer at CDC Office of Blood, Organ, and Other Tissue Safety.

DR. JACKSON: Thank you all for coming.

Bryan, will you please read the conflict-of-interest statement?

Agenda Item: Conflict of Interest Statement

LCDR EMERY: I’m Lieutenant Commander Bryan Emery. I’m the designated federal official for this meeting of the Blood Products Advisory Committee. I will start with the FDA conflict-of-interest disclosure statement.

The Food and Drug Administration is convening May 13, 2015, for a meeting of the Blood Products Advisory Committee under the authority of the Federal Advisory Act of 1972. With the exception of the industry representative, all participants of the committee are special government employees or regular federal employees from other agencies and are subject to federal conflict-of-interest laws and regulations. The following information on the status of the Advisory Committee’s compliance with federal ethics and conflict-of-interest laws, including but not limited to 18 US Code 208, are being provided to participants at this meeting and to the public.

FDA has determined that all members of the Advisory Committee are in compliance with federal ethics and conflict-of-interest laws under 18 US Code 208. Congress has authorized the DA to grant waivers to special government employees and regular government employees who have financial conflicts when it is determined that the agency’s need for a particular individual’s service outweighs his or her potential financial conflict of interest.

Related to the discussions at this meeting, members and consultants of this committee have been screened for potential financial conflict of interest of their own, as well as those imputed to them, including those of their spouse or minor children, and for the purposes of 18 US Code 208, their employers. These interests may include investments, consulting, expert witness testimony, contracts and grants, CRADAs, teaching, speaking, writing, patents and royalties, and primary employment.

For Topic I, the committee will discuss strategies for implementation of serological nucleic acid testing for *Babesia microti* in blood donors. This is a particular matter of general applicability.

For Topic II, update regarding the FDA’s consideration for a revised blood donor deferral policy for men who have sex with men, this is a particular matter of general applicability.

Topic III: In closed session, the review of the research programs in the Laboratory of Cellular Hematology, Division of Hematology, OBRR. This is a non-particular matter.

In addition to the committee discussions, the committee will hear updates on considerations for hemoglobin S testing in blood donors, considerations for a revisable donor deferral policy for men who have sex with men.

Based on the agenda and all financial interests reported by members and consultants, no conflict-of-interest waivers were issued under 18 US Code 208. Dr. Toby Simon will serve as the industry representative. Dr. Simon is employed by CSL Behring of King of Prussia, Pennsylvania. Industry representatives act on behalf of all related industry. Industry representatives are not special government employees and do not vote.

There may be regulated industry speakers and other outside organization speakers making presentations. These speakers may have financial interests associated with their employer and with other regulated firms. The FDA asks, in the interest of fairness, that they address any current or previous financial involvement with any firm whose product they may wish to comment upon. These individuals were not screened by the FDA for conflict of interest.

We would like to remind members, consultants, and participants that if the discussions involve any other products or firms not already on the agenda for which the

FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement, and their exclusion will be noted for the record.

FDA encourages all other participants to advise the committee of any financial relationships that you may have with any firms, its products, and, if know, its direct competitors.

This conflict-of-interest statement will be available for review at the registration table.

This concludes the reading of the COI statement for the record.

For today’s meeting, participants are also being provided with the following guidance: The May 13, 2015 BPAC meeting is a particular matter of general applicability and, as such, does not focus its discussion on any particular product, but, instead, focuses on various strategies and methodologies -- serological and nucleic acid testing, for example -- for implementation of *Babesia microti* in US blood donors. This BPAC meeting is not being convened to recommend any action against or for the approval of any specific serological and nucleic testing products for the screening of *B. microti* in US blood donors. This BPAC is not being convened to make specific recommendations that may potentially impact any specific product, party, entity, or firm in a unique way. Presenters and speakers will provide data on the investigational blood donor screening for *B. microti* using serological and nucleic acid screening methods that serve only as examples for the committee to have a scientific discussion while considering various classes of technologies as screening tools.

This BPAC meeting will not involve the approval, disapproval, labeling requirements, postmarketing requirements, or related issues regarding the legal status of any specific products or methods, and any discussion of individual products and methods will be only to serve as an example of the product class.

The BPAC committee consumer representative, Mr. Corey Dubin, was unable to attend today’s meeting. Judith Baker is the appointed consumer representative as his replacement.

Dr. John Holcomb, our surgeon, was unable to attend today because of travel difficulties related to weather conditions in Texas.

Tara Goodin is standing in the back. She is the FDA press representative. If you have questions, you can see Tara for that.

Please remember to turn off or set to vibrate all your cell phones and electronic devices.

At this time, I will turn the meeting over to the chair, Dr. Brooks Jackson.

DR. JACKSON: Thank you very much, Bryan.

Before we start, we had a couple members join in the last couple of minutes. Could you just introduce yourselves?

DR. LERNER: Norma Lerner, NHLBI.

DR. LEITMAN: Susan Leitman, NIH.

DR. JACKSON: Thank you.

Our first topic, as you just heard, is on strategies for implementation of serological and nucleic acid testing for *Babesia microti* in blood donations. Our first speaker is Sanjai Kumar. Dr. Kumar will give the introduction and background on this topic.

Agenda Item: Topic I: Strategies for Implementation of Serological and Nucleic Acid Testing for *Babesia microti* in Blood Donations

Introduction and Background

DR. KUMAR: Good morning, everyone. Thank you, Dr. Jackson.

As Dr. Jackson said, I am going to introduce this topic before the committee today: Strategies for implementation of antibody and nucleic acid-based testing for *Babesia microti* in blood donations.

Why this issue has come to the fore this time?

Babesia microti is among the most frequently transfusion-transmitted infections reported to FDA, with associated fatalities, for which no donor testing is available. So this remains one of the biggest unsolved cases. Risk of acquiring *Babesia* infection by blood transfusion is nationwide, even though the transmission is local in states, localized areas. Recent investigational testing of blood donations for *Babesia microti* infection provides data on the potential utility of testing. We will hear data on this during the day.

What are the major issues? FDA is seeking advice on donor testing strategies for evidence of *Babesia microti* infection. The basic elements boil down to these three issues here:

- Should antibody testing be nationwide and year-round?
- Should nucleic acid testing, or NAT, be limited to certain high-risk states?
- Should alternative approaches be also considered based on geographic and seasonal risk?

With that, I'm going to give a brief summary of *Babesia microti* life cycle:

- Enzootic transmission. That means the transmission in endemic areas in the United States for *Babesia microti* -- that is the only one I will be discussing here today -- is self-sustained and is sustained in the absence of outside forces.

• Sylvatic reservoir. In this case, it is this white-footed *Peromyscus* mouse, the erythrocytic asexual cycle. Then there is the invertebrate *Ixodes* tick vector that supports a sexual cycle and transmits the infection. Deer, in this case white-tailed deer, provide the blood meal and transport to ticks.

• Human is the incidental host, and dead-end host.

• These chronically infected asymptomatic individuals are the ones which are the matter of concern in transfusion-transmitted babesiosis.

Looking at the *Babesia* species, those are prevalent in the United States. *Babesia microti* is the predominant species. The highest clinical burden and transfusion-transmitted cases are from *Babesia microti* -- you will see some of these numbers later on -- and our biggest concern. *Babesia microti* is mostly localized in the Northeastern states and Mid-Atlantic. There are now around nine or ten states where *Babesia microti* is transmitted. There are two Midwestern states, Wisconsin and Minnesota.

Looking at non-*microti* species, in the Pacific Northern states, *Babesia duncani* is transmitted in California and in Washington State, and then a few cases are sporadically reported for *Babesia duncani*-type organisms, along with *duncani* reported from Washington State, and then *divergens*-like parasite, which is a closer cousin to a European counterpart, is reported from Kentucky and Missouri.

But the important thing to remember here today is that assays that are designed for *Babesia microti* may fail to detect the other *Babesia* species prevalent in the United States. We have to keep that in mind throughout the presentation.

Epidemiology of babesiosis: You will hear about this a lot more from Dr. Herwaldt and perhaps in other presentations. As I said in the previous slide, endemic transmission is reported mostly in the Northeast, Mid-Atlantic, and Upper Midwestern states. But the area of endemic transmission is reported to be expanding, particularly to those states that are adjoining the endemic states. Several other states without recognized endemic areas also report babesiosis cases -- again, you will see the data on that -- due to infections acquired during travel to endemic areas. That is primarily the cause where babesiosis is transmitted where the disease is not endemic.

Clinical symptoms and pathogenesis: I will just touch very quickly on this because you will hear more details later.

• Ranges from asymptomatic, silent clinical infection to mild disease, which more feels like viral infection, to life-threatening severe disease.

• A majority of individuals infected with babesiosis may remain clinically silent.

• Neonates, immunocompromised, asplenic, and elderly are at the highest risk of severe disease.

• Clinical symptoms generally one to four weeks after a bite by an infected tick, although aberrations are reported.

• Fatality rates among those severe cases which require hospitalization are around 6 to 9 percent, and in the case of immunocompromised, disease is more severe and fatal. Around 20 percent of those may die.

What are the major available data sources to assess babesiosis risk in the United States? The first one is the National Babesiosis Surveillance Program that was started in 2011. Since then, solid data for three years has become available, clinical cases reported to CDC. Transfusion-transmitted babesiosis cases Dr. Herwaldt has published a very seminal paper on, 2009, that reports all cases which were transfusion infection, 1979 to 2009. After that, from 2010, we relied on the data from biological division reports to FDA. That is up to more recent, from 2014. The biggest data source is the national surveillance for Center for Medicare and Medicaid Services, CMS, health records for beneficiary claims for diagnosis of babesiosis in persons 65 and older. This is for eight years, 2006 to 2013. You will hear a lot about this from more colleagues from FDA.

Clinical babesiosis cases represented by state: It’s important to mention that even if it is nationally notifiable, not all the states participate. So far 31 states have participated, and the disease has been reported from 36 states; in 2013, clinical cases reported from 22 states, around 1,800 cases, around 98 percent of these cases from the known nine endemic states that I referred to earlier. That is where most disease burden is. But a few believe this is like the tip of the iceberg because there is huge underreporting due to misdiagnosis or non-diagnosis of both clinical cases and asymptomatic infections. So disease is perhaps far more prevalent with higher disease burden.

Transfusion-transmitted babesiosis was first reported in 1979. Again, you will hear more detail from Dr. Herwaldt. Through 2014, if you take cases reported both to CDC and to FDA, there are certainly more than 225 transfusion-transmitted infections and 28 deaths associated with this. As far as we know, out of all these cases, only three cases can be attributed to *Babesia duncani* and the rest are *Babesia microti*. So that is where the burden is. On average, putting it in simpler terms, we see about 15, more or less, transfusion-transmitted babesiosis cases each year in recent years. So that is the current burden.

Clinical presentations of transfusion babesiosis:

- It can be subclinical to severe disease, depending on the health status of the transfusion recipient.
- Incubation period of one to nine weeks, but it may be delayed for up to six months, as reported.
- Fatality has been reported from 6 to 20 percent. Again it depends on the health status of recipient.
- Signs and symptoms of clinical disease resemble those of tick-bite infections.

Distribution of transfusion-transmitted babesiosis by state: There are 205 cases out of the 225 total cases which we know of for whom the state of donation is known, reported from 22 states. Again, even if a natural transmission of *B. microti* may be restricted to nine states, transfusion infections occur in far wider ranges. About 87 percent of those cases are in nine endemic states. So the clinical disease and transfusion infections both coincide.

Nationwide prevalence of babesiosis, as assessed from CMS data: During the eight-year period, more than 10,000 unique diagnoses of babesiosis were detected from the data set. The cases were reported from all 50 states, including Washington, DC, except for the state of Wyoming. That shows that the burden of disease is nationwide.

Babesia transmission is regional while transfusion infection risk is systemic. That is the major issue here. It’s nationwide because the donors from non-endemic areas travel to endemic areas and acquire infection. Donors who normally reside in endemic areas may donate elsewhere. I think our friends from the blood centers, blood industry, can tell us more about it, but we know the interstate blood commerce -- blood products are often shipped between widely separated regions across the United States. Therefore, we think that screening is needed where blood is collected. That is an important point to keep in mind.

Tick-borne transmission is seasonal, but transfusion-transmitted infection risk is year-round. Around 80 percent of clinical cases were reported between the months of June and August. That is the peak transmission season. However, transfusion-induced infections continue to occur year-round, attributable to chronic asymptomatic infections.

I was looking at Dr. Herwaldt's paper, and at one point it is reported that around 59 percent of transfusion-transmitted infections were actually due to donations collected in July through October. So there is a slight disconnect here. While clinical cases happen, 80 percent, here, transfusion infections occur, around 40 percent, out of this peak transmission season. Those directly can be related because the clinical disease -- asymptomatic donors keep causing transfusion infections outside the transmission season. This is data to support that contention.

Also we know that both parasitemic donors and/or antibody-positive donors are identified year-round.

Blood components that cause transfusion infections: It has been caused by red blood cells and whole blood-derived platelets. In infected red cells the parasite can survive under refrigeration for the entire shelf-life of blood. Cryopreserved RBCs can cause transfusion infection. Leukocyte reduction and irradiation are not effective. The effectiveness of pathogen-reduction technology against *Babesia microti* in whole blood and RBCs has not been established.

When it comes to the issue of strategies for implementation, basically we are talking about when to test, where to test, and which test to use. For that, we need a better understanding of the parasite biology and host response. We have constructed this model of *Babesia microti* infection in a healthy host. I must say, there are inherent uncertainties and variability in this model.

Basically, the reports, as they become more and more available -- usual infections last for three to five months. Then you have this early phase when the parasitemia is rapidly rising in a host. There is a time of replication after infectious tick bite where the threshold is too low to be detectable by a nucleic acid test. But then again, this time is going to vary depending on the host’s immune status, health status, and the replication time. Then the parasitemia arises. Then sometime around five months to six months, it comes down. It becomes non-detectable. In some individuals, for reasons we don't understand, it establishes as a protracted chronic infection. These are the chronic donors which are the major concern. These infections can last up to a year, two years, in one case up to 27 months. These are chronic infections here.

Looking at the immune response, antibody response, detectable antibodies begin to appear sometime around three months, three weeks post emergence of parasites. They peak around a month or two after the antibody, parasitemia, and then they come down, in a subset of individuals, about a year after. You will see data hopefully from Dr. Sue Stramer. But in some individuals, antibodies have longer longevity. They can go on to persist for a year, two years, and even three years. Whether the same donors who are asymptomatic carriers maintain this high antibody titer we don't understand fully now.

What are the issues in addressing transfusion-transmitted babesiosis? Many of these I have already discussed in my previous slides:

• Both clinical cases and TTB cases are underreported.

• Asymptomatic carriers are thought to be the primary source of transfusion-transmitted babesiosis.

• *Babesia* infection can persist with low-grade, asymptomatic parasitemia for months to greater than two years.

• The minimum infectious dose that can cause transfusion infection is not known.

• Parasite burdens in asymptomatic carriers are not known.

These two relate to the issue of the detection by nucleic acid testing, whether the most sensitive assays may even miss some infections.

• There is no recommendation to treat asymptomatic babesiosis. That is how the pool of infected donors is maintained in the donor population.

Looking at the particular issues of antibody testing, which have implications for its implementation:

• Antibodies, if highly sensitive assay are sensitive enough, expected to detect the vast majority of parasitemic donations.

• Can detect low-grade, chronic infections that may be missed by nucleic acid testing.

• Will fail to detect infectivity prior to seroconversion. I was trying to show that in this slide earlier.

• Presence of antibody does not necessarily indicate active infection.

• Seroreversion, in the absence of chronic infection or new infection, may allow donor reentry based on negative antibody test results -- another important point to keep in mind.

Issues in nucleic acid testing:

• Can detect window-period infections prior to seroconversion.

• More suitable for areas of active tick-borne transmission -- that is, endemic areas.

• May fail to detect low-grade chronic infections and early window-period cases. That again relates to the sampling issues and the parasite threshold.

• Has limited ability to differentiate between active and cleared infections. The reason I say limited ability is because what we may consider cleared infections may be below threshold of detection and may not repeat by nucleic acid testing.

In the past, we had visited this issue with the Blood Products Advisory Committee. In July of 2010 we discussed this issue of a risk of *Babesia* infection by blood transfusion and potential strategies for donor testing. The question that we had posed to the committee at that time was whether, based on FDA risk analysis -- we asked whether a certain number of the states for the concept of regional testing of blood donations for *Babesia* infections. That was the question, whether it's okay to perform regional testing for blood donors for *Babesia* infection. Then we had two other questions: benefits of broad-based regional testing of blood donors by nucleic acid testing and suitability of antibody testing for *Babesia* infections in blood donors.

The committee did at that time unanimously endorse the concept of regional testing of blood donors for *Babesia microti*.

What has changed by now: Investigational testing of donations for *Babesia microti* has been conducted by two manufacturers. You will hear about their assays and clinical performance of their assays later in the day.

IMUGEN has a PCR-based test for detection of *Babesia microti* DNA, and then a test for detection of anti-*B. microti* antibody. Immunetics has an enzyme immunoassay-based test for detection of *B. microti* antibody.

Then we have this FDA risk model to assess babesiosis risk throughout the country. My colleagues, Dr. Mikhail Menis and Richard Forshee, will tell you about this a lot more, but just to get you introduced to the model, it’s based on the CMS data set to assess the baseline potential risk of babesiosis in US blood donors. They are going to show you the data on the potential risk reduction under various testing scenarios -- one is the antibody-only testing in selected states or nationwide testing by antibody, testing with both antibody and nucleic testing in selected states or nationwide -- potential blood units lost due to false-positive test results, an important consideration, positive selective value of testing for markers of infection. Then they also take into consideration sensitivity analysis to examine variable infectivity of positive units.

Just very quickly, the agenda that follows after this presentation: Dr. Herwaldt will tell about the epidemiology of babesiosis. Then Dr. Jeffry McCullough will talk about considerations in transfusion-transmitted *Babesia microti*, from the University of Minnesota. Dr.

Menis and Dr. Forshee will present results of the FDA risk model and experiences with the investigational testing of blood donors for *B. microti*, Dr. Stramer from the American Red Cross will be representing IMUGEN, and Dr. Andrew Levin will present his own data, from Immunetics.

Then I will be back one more time presenting the considerations for testing of blood donations for *B. microti*, basically initiating the discussion. Open public hearing, open public discussion -- we will have presentations here, then questions for the committee.

I would just like to leave you with the question -- the first question, so you have something to ponder about while you listen to the rest of the questions -- and you have the questions in your packet also -- do the available scientific data and FDA analysis support the concept of nationwide, year-round testing of blood donations for *Babesia* risk by an antibody-based test? If not, please comment on alternative options that FDA should consider, including limitation of antibody testing to specific states.

The second question relates to nucleic acid testing. Does the committee agree that NAT-based testing should be performed in blood donations in certain high-risk states? If so, please advise whether year-round NAT testing should be considered in the following. There are three options of states here. The first one is five states. These are the highest endemic states where the most disease burden lies: Connecticut, Massachusetts, Rhode Island, New York, and New Jersey. The next one is the nine-state option. These are all known endemic states, the first five states from here and two other states, Minnesota and Wisconsin -- these constitute the historic seven endemic hottest states -- and then the state of New Hampshire and Maine. The next strategy is the 15-state, which is a more expansive strategy, 15 states plus Washington, DC. They are the highest risk captured with the smallest number of states. These states are listed here: Connecticut, Massachusetts, Rhode Island, New Jersey, Minnesota, Wisconsin, New Hampshire, Maine, Maryland, Washington, DC, Virginia, Vermont, Pennsylvania, Delaware, and Florida.

Also please comment on alternative options that FDA should consider for blood donation by NAT.

The third question: Please comment whether it would be appropriate to apply a time-based deferral for those donors who have *B. microti*-positive test results. If so, please advise on a suitable deferral period for donors who had *Babesia microti*-positive test results.

With that, I will stop here and we will proceed with the next presentation, I guess. Thank you.

DR. JACKSON: Thank you very much, Dr. Kumar.

Our next speaker will be Dr. Barbara Herwaldt, from the Centers for Disease Control, who will present on the epidemiology of babesiosis.

Agenda Item: Epidemiology of Babesiosis, Including Transfusion-Associated Infection.

DR. HERWALDT: Thank you very much. It’s an honor to be here today.

I am going to be whizzing through a fair number of slides that will have lots of numbers on them, but my focus is on big-picture principles and perspective. And, of course, I have to include the requisite disclaimer.

I typically like to underscore as one of my themes throughout talks the temporal and spatial dimensions. That theme will come up over and over again, including the changes over time.

I typically like to start with some historical perspective. I’m not starting as far back in time as I usually do. I’m starting now in 1956, the first documented zoonotic case of babesiosis, which occurred in the former Yugoslavia. Before I go on, I want to remember to say that obviously there is a big difference between “documented” and “occurred,” but this is the first well-documented case. Whenever I provide a year, it is the year in which something occurred, not the year in which it was published. Later on, when I talk about transfusion-associated cases, those will be stratified or classified by year of transfusion and state of transfusion.

So 1956 for the first well-documented zoonotic *Babesia* case. Then ten years later, 1966, the first documented zoonotic US case. Believe it or not, it was on the West Coast, in California. The species was not known or identified at the time and can’t be determined in retrospect. Three years later, the first documented US *B. microti* case, on Nantucket Island in Massachusetts. This was when Nixon was president, to orient you in time. Then in 1979, when Carter was president -- so ten years later -- the first described US transfusion-associated case, which was caused by *B. microti*, occurred in Massachusetts and was linked to whole blood-derived platelets.

The first BPAC meeting that was convened that addressed babesiosis was in 2010. And here we are today, 2015, with the second BPAC meeting regarding babesiosis.

Some basic perspectives, some of which Dr. Kumar has already alluded to. As you all know, babesiosis is a potentially life-threatening zoonosis caused by parasites that live inside red blood cells. In the US, as you know, the main etiologic agent is *Babesia microti*, which is transmitted by tiny *Ixodes scapularis* ticks, so tiny that they are easily missed. But it is also transmissible by transfusion, and small inocula may suffice. Seasonal tick-borne transmission of *B. microti* occurs in evolving foci of endemicity in what I loosely refer to as the Northeast, especially in foci in New England, New York, and New Jersey, but also in other foci. For example, there are well-established endemic foci now in Pennsylvania, particularly in eastern Pennsylvania, also in the Upper Midwest, in parts of Minnesota and Wisconsin, as you already know.

I like to show this slide to remind people again that transmission is dynamic in time and place. There can be good years or bad years for ticks. Whether you call them a good year or a bad year depends on your perspective about ticks. Obviously, from our perspective, we don't like to have a lot of ticks around. Where are people in this picture? They are not in this picture, obviously, because people are not normally part of the transmission cycle.

In terms of clinical aspects -- again, principles and perspective -- regardless of the route of transmission, *Babesia* infection can range from asymptomatic to severe. Regardless of their severity, manifestations are nonspecific. Diagnostic testing is required. Of course, as all of you know, diagnostic testing is very different than donor screening. What I’m talking about now is diagnostic testing. Actually, I am not going to go into any details about laboratory issues.

Symptoms, if any, usually develop within several weeks or months of the exposure, but they may appear much later. For example, you could have subclinical or latent infection that becomes clinically manifest after a splenectomy.

Risk factors for clinically manifest infection versus asymptomatic infection and for severe cases of infection include asplenia, advanced age, with various criteria for what should be classified as advanced age, and various other causes of what I am very loosely referring to here as immune dysfunction. Again, I’m loosely referring here to immunosuppressed patients. They may be afebrile and/or they may have subacute, remitting-relapsing clinical courses.

To underscore the importance of the spleen, I like to show this slide, reiterating over and over again the importance of the spleen, which does not mean that everyone without a spleen or without a functional spleen develops a severe case, nor does it mean that this is the only risk factor. But it is a very important one to keep in mind.

Additional perspective: Even asymptomatic *Babesia* infection can be associated with low-level, subpatent -- in other words, not detected by blood smear examination -- parasitemia for weeks to months, sometimes longer than a year or two, probably not lifelong. The risk factors for protracted infection in otherwise immunocompetent persons have not been elucidated. Subpatent parasitemia sets the stage for transmission via transfusion. Protracted infection sets the stage for year-round transmission. As you know, people can meet all of the current criteria for donating blood despite being infected and infective.

Effective January 2011, babesiosis was designated a nationally notifiable condition, with a standard case definition for surveillance purposes. It’s not a clinical case definition per se. In other words, it’s not meant for diagnosing a case in an individual person. It was designated a nationally notifiable condition to enhance the ability of public health authorities to detect, monitor, and prevent cases.

I’m going to be showing you some case counts, but as with all case counts, remember the caveats. I’m not going to go into the details of the case criteria or the logistics of surveillance. But suffice it to say that for all case counts, including the transfusion-associated case counts I will be providing later -- but right now for the surveillance case counts -- always consider how, where, when, and by whom were cases detected, investigated, classified, reported, and analyzed, for what purposes. Obviously cases have to be in persons who seek medical attention, have an appropriate diagnosis, and the cases are reported and classified and counted.

For the years 2011, 2012, and 2013, I’m going to provide the data here. In 2010, as you know, babesiosis was not yet a nationally notifiable disease. The data for 2014 have not yet been finalized, but they are relatively comparable to the data, in aggregate, for 2013. These case counts -- 2011, 1,126; a bit lower, 911, in 2012; and then 1,762 for 2013 -- they slightly differ from those in the MMWR summaries. These case counts are not affected by the fact that over time babesiosis has become reportable in additional states. I will show more in that regard in a moment.

In particular, in this slide, which shows the number of reported cases by year for those three years, in different colors, for the seven states that each, in total, notified CDC of more than 150 cases -- and these seven states accounted for 96 percent of the reported cases for this three-year period -- you can see that, for example, in New York there was a dip down in 2012; in Massachusetts, each year somewhat more. As Al DeMaria can tell you, in 2014, although the data aren’t here on this slide -- again, they have not yet been officially finalized for the country as a whole -- the case count is higher. But it varies from state to state over time. Even for Lyme disease, there was a dip, at the national level at least, in 2012 in terms of case counts.

So case counts can vary for both real reasons -- bad tick year, good tick year -- and also for artifactual reasons.

I'm going to show a couple maps on this slide, not with high enough resolution for you to see the details, but it is more to show big-picture perspective. These maps have numbers. It is not incidence. It is numbers of reported cases by county of residence, not necessarily county of exposure, contrasting 2011 with 2013. In any state that is in gray, babesiosis was not a reportable condition. You can see that not only are a lot of the states in which it was reportable in the Northeast and Upper Midwest, but that is where there is a lot of orange and dark orange. In 2011, county of residence was unknown for about eight of the cases. But you see that there were 20 states in which babesiosis was a reportable condition. Seventeen of those states reported at least one case.

In 2013, you can see that there are fewer gray states. The number of states in which babesiosis was reportable was up to 27. In 2014, it's up to 41, and for 2015, it's a bit higher. The case counts are a bit higher. You can see that there is darker orange in the Northeast. By the way, I already told you that this is by county of residence, not necessarily county of exposure, but this slide serves as a reminder that babesiosis is endemic in foci in Maine. Also you can see in the Upper Midwest -- I don't know if this is real or artifactual -- that there are more counties that have reported cases.

This shows, for the cases for whom information was available, distribution by symptom onset. You can see that, although all months had some cases with symptom onset -- these are for case patients, of course -- the bulk were in the several-month period. All of these case patients had to have a symptom onset date, so these are for clinically manifest cases.

For distribution by age group, you can see it skewed towards older persons. This is a very different age distribution than, for example, Lyme disease. There may be a number of explanations for different clinical manifestations and different rates of clinically manifest infection in younger children who are otherwise immunocompetent versus in older persons.

Tick-borne transmission unfortunately can secondarily lead to transfusion transmission, which is why we are here today. The rest of my talk will focus on transfusion-associated babesiosis. This also serves as a reminder or an opportunity for me to put in some prevention messages. Here are some bookmarks telling people that your to-do list is not complete without a tick check, assuming you live in a relevant area. Remember to do your tick checks.

In terms of transfusion-associated babesiosis, I’m going to be talking about some of the case counts and analyses of data for what was a very collaborative effort, including multiple people besides those on the authorship list. It, in fact, included many of you. “Transfusion-associated babesiosis in the United States: a description of cases,” this was published in the year 2011. It covered a 31-year period, from 1979, the year, as I already told you, of the first described US transfusion case, through 2009.

Here is that 31-year period. I’m going to be mentioning the 159 *B. microti* cases that came to our attention and met our criteria -- again, always remember the nuances and the caveats for case counts -- and three *B. duncani* cases. For 2010 to 2014-2015, the case counts haven’t yet been finalized. Of course, they have been finalized for some individual states and for individual blood collection agencies. Certainly it’s fair to say that there have been more than 200 total identified cases.

For the cases counted in the article I referred to -- the purpose of this slide is not for you to get bogged down in the numbers, but rather just to give you a perspective about why there are different denominators that I will be referring to. Overall, 162 cases, three caused by *B. duncani*, which I will just briefly refer to in the next slide, then 159 caused by *B. microti*. Because there were some cluster-associated cases, that necessitated distinguishing index cases from non-index cases, which is why the rest of the slide exists.

There were 12 clusters, with a total of 30 cluster cases. Each of those clusters had one index case and then the other cases were in, for example, co-recipients or persons who were identified by look-back or look-forward investigations. All of the other case patients were defined as index case patients. That’s why there is the distinction between 162, 159, and 141.

A very brief aside. Understandably, *B. microti* is the highest priority now, and it should be. But don't forget that there are other species, including *B. duncani*, the WA1-type parasite, which are not detectable, as you know, by serologic or molecular testing for *B. microti* per se. As already alluded to, there have been three documented *B. duncani* cases. In addition, my colleagues and I documented a window-period donation in another Western state. The documented *B. duncani* cases have been in Washington and California, and the window-period donation was documented in Oregon in someone who had donated blood about a week before he became clinically ill. The blood had not yet been transfused. So after he sought medical attention, the unit was sent to CDC. We documented by animal inoculation the presence of the parasite and documented that was *B. duncani* by molecular testing, and then also documented seroconversion. The patient had been seronegative and became seropositive when he became clinically ill.

Now back to *B. microti*. This slide shows the 159 cases by year of transfusion and shows the ups and downs over time -- overall, more cases in the last decade. It’s also showing, in case you are interested, the distinction between index cases and non-index cases. Of course, the non-index cases aren’t independent, because they were part of clusters.

The bulk and the range are important. For the 159 cases -- and I keep emphasizing, because it’s so important, that these are cases that came to our attention through various means and met our criteria to be defined as transfusion-associated -- the case patients’ median age was 65, but 32 percent were either very young -- 18 were infants, many of whom were premature -- or very old. Thirty-three were in their ninth or tenth decade of life.

The cases -- and again these are the transfusions, because they were stratified by state and year of transfusion -- occurred in all four seasons and in 22 of the 31 years. But 77 percent of the identified cases occurred during the third decade. That could be increased risk. It could also be increased awareness. It could be all sorts of things. The transfusions occurred in 19 states -- if we go forward in time up to 2014-2015, the number of states is up to the mid-20s, but this is for 1979 to 2009, 19 states -- but 87 percent occurred in the seven states defined for purposes of the analyses as the main *B. microti*-endemic states. This is not the entire list of *B. microti*-endemic states. It doesn't include Pennsylvania in this list or Maine, but it's just to give you some sense. By the way, the percentages are the same both here and here, regardless of whether it is total cases or index cases.

Most cases were associated with red blood cell components, even if they were old or leukoreduced, irradiated, or frozen/deglycerolized. Four cases were linked to whole blood-derived platelets, presumably because of residual red cells and parasites.

This shows by index cases, by period -- this is a six-year period, and these are five-year periods -- it shows that for the third decade more cases were identified. It also differentiates between -- this is an overly simplistic differentiation -- cases for whom the transfusions were in so-called babesiosis-endemic states -- this is with the traditional seven-state definition -- versus in other states.

Now, *B. microti* has caused transfusion-associated cases in what I call far-flung states in the context of interstate travel of donors and blood components. In essence, they circulate. So we have babesiosis without borders, but not equal risk.

These are just some examples -- it is not a complete list -- of geographic outliers, either during the 1979 to 2009 period or thereafter. Anytime I say something is a recent case, I mean it is since 2010:

• California, two documented cases, one linked to a donation in Maine. One, a recent case, a California resident donated blood after having traveled to the Northeast, which is a recurring theme.

• Washington, two *B. microti* cases. Again, these are all *B. microti*. A Rhode Island resident donated blood in Washington while training there. A recent case: A Washington resident donated blood after having traveled to the Northeast. As I already said, these are *B. microti* cases.

• Texas, two cases, one linked to a donation in New Jersey. I have highlighted in yellow if there was a donation in a state in what I'm loosely defining as the

Northeast. A Texas resident donated blood after having summered in the Northeast.

• Florida, three cases. A Wisconsin resident donated blood in Florida while wintering there. A Florida resident donated blood after having traveled to the Northeast. In one case, don't know; donor was not identified.

Which reminds me to say that no cases were counted in the paper I discussed if all the donors had been tested and they all tested negative. But in situations in which not all donors had been tested, but it was pretty clear that transfusion was the route of transmission, there were some such cases counted.

• In Georgia and the Carolinas, a recent Georgia case. A Georgia resident donated blood after having traveled to the Northeast. There were lots of types of travel associated with this case. The recipient actually was from the Midwest, not from a babesiosis-endemic state. That’s where the diagnosis was made, in the Midwest. But that recipient had traveled way down to Georgia to have surgery and to have a blood transfusion. The donor was a Georgia donor who had traveled to the Northeast. Then the recipient left Georgia, went back to the non-*B. microti*-endemic state in the Midwest, and the diagnosis was made there, again underscoring the importance of awareness among clinicians and the blood community.

A recent South Carolina case linked to a donation in Connecticut. A North Carolina case for whom a donor was not identified.

Remember, I said these are just examples. In addition, donor travel has been associated with cases in Illinois, a recent case, Indiana, Maryland, Ohio, Virginia, and blood shipment and blood distribution have been linked to cases in various outlier states.

Seasonality: Distribution by month of the blood donations associated with US *B. microti* cases. The month of donation was known or estimable for 128 of 148 donations by 141 donors. Some donors donated more than once because of the cluster-associated cases. You can see that there is a seasonal peak, but only in relative terms.

So transmission in all four seasons, all 12 months.

In closing, just to reiterate, donor travel to and from foci of endemicity, unrecognized protracted infection in some donors, intraregional distribution and interregional shipment of blood translate into the potential -- not equal risk, but the potential -- for transmission by transfusion anytime, anywhere.

Again, upward trend? No definitive conclusions can be reached about the seeming upward trend in the numbers of transfusion-associated cases that came to our attention and met our criteria, but cases have continued to be documented.

I like to refer to “the tip of the blood-berg bag.” Undoubtedly, the cases that have been recognized represent a fraction of those that have occurred, but the sizes of the relative fractions, of course, aren’t known.

But there is typically a story. Even in babesiosis heartland -- there are some experts here from Massachusetts -- even in the best-case scenario where people are very knowledgeable about *Babesia microti* infection, often a patient is severely ill before coming to medical attention and having the diagnosis made. I’m not picking on Massachusetts. I’m just saying that it is not just a phenomenon in states in which babesiosis is not endemic. Often there is a series of serendipities. Even severe cases often are serendipitously detected.

My last slide. I often end with the question, whither *Babesia*? Keep your eye on this tetrad. Of course, we wish it would just go away, but unfortunately we have the questions of what to do, where to do it, and how to do it.

Thank you very much.

DR. JACKSON: Thank you very much, Dr. Herwaldt.

Our next speaker is Dr. Jeffrey McCullough, from the University of Minnesota, who will speak on considerations in transfusion-transmitted *Babesia*.

Agenda Item: Considerations in Transfusion-Transmitted *Babesia microti*

DR. MCCULLOUGH: I pull up the chair because I had lumbar surgery a couple weeks ago, and my one leg occasionally gives out unexpectedly.

I’m going to take a little different approach from the previous speakers in focusing on patients in the hospital situation. Presuming that the *Babesia*-tested blood becomes available, then our issue is, which kinds of patients should receive tested blood?

What you are going to see here is work much of which was done by one of our former fellows, Deanna Fang, who also had a fellowship in medical microbiology. She was perfect to do this.

The theme of what I’m going to show you is that transfusion-transmitted babesiosis affects a wide variety of patients.

This is my disclosure. As far as I know, I’m not going to say anything about Fresenius Kabi, but I’m obligated to show you this, regardless.

What Dr. Fang did was an extensive literature search. You may see some numbers that will be different from the numbers you have seen in the previous slides, because what we are focusing on is published cases from either case reports or abstracts. There will be some cases that the CDC has that have never been published, so they won't be in the data that I am going to show you.

Deanna used the search term "babesiosis and transfusion," "*Babesia* and transfusion," and up to August of 2014. She also searched AABB annual meeting abstracts, the FDA's "Fatalities Following Blood Collection," references to other references, and personal communication.

The inclusion criteria were *B. microti* only and transfusion-transmitted cases occurring within the United States. Exclusion was non-human cases, non-*microti* species, and cases occurring outside the United States. This resulted in 65 different publications, either full publications or abstracts. That forms the basis of the data that I am going to show you.

These were included regardless of the recipient's outcome. Ten cases may or may not be multiply reported. There wasn't enough information to determine that, so we have included those ten cases. The FDA also on their website has four cases that reported after 2010, but aren't described elsewhere in the literature, so we did not include those.

I'm going to be talking about a total of 256 cases. In 241 of those, the donors were known to be test-positive, and in an additional 34, it’s pretty clear the donors would have been test-positive based on what was said in the case reports. In 15 cases, there was pretty clearly transfusion-transmitted *Babesia*, but the donor either was not identified or details of the donor’s testing were not provided in the literature. So 256 is the number that you are going to see in most of the rest of these slides.

What does this represent? In 165 of these, the recipient tested positive. In 15 of them, the donor’s test results were not reported. In 60 cases where the donor was positive, the recipient tested negative. There are 31 other cases in which the donor was positive and the recipient’s test was not clear from the case report.

Deanna and I developed six different categories of disease severity in these patients. Class 0, given here, is patients in which the donor was positive, but the recipient was known to test negative. Category 1 is recipients were positive, but asymptomatic. Category 2, recipients were positive, but the transfusion-transmitted disease was uncomplicated, treated with antibiotics alone. Category 3 was patients who were symptomatic and complicated. They developed other related problems, possibly ventilation, dialysis, exchange transfusion, those sorts of complications. Category 4 is patients who died. Category 5 is patients who we were unable to categorize based on the kind of data that was available in the reports.

This is a graphic illustration of what happened to those patients. You will see that the same color scheme will be used in subsequent slides. On your left, the blue is the 60 patients who did not appear to become infected. On the far right are 106 patients that we were unable to categorize. You can see the mixture of patients in the other categories, from asymptomatic to death. Of the 256 cases that we identified, 38 of them resulted in fatality.

Now, one of the problems with this data is that it is very difficult to determine from these case reports the role of *Babesia* in death. Some of these patients pretty clearly died of other causes, although it’s likely that *Babesia* didn’t help them along. Some of them also seemed to die of the *Babesia*, but it’s very difficult. We just lumped all the patients who died of the 256 that we identified.

This is a partial list of what the infectious disease textbooks say are classic risk factors for babesiosis: splenectomy, cancer, HIV, hemoglobinopathy, chronic heart and lung disease, neonates, so on.

We then tried to identify what we thought was the most underlying medical condition for these 256 patients so that we could relate the *Babesia* to one disease category. Unfortunately, as you see on the far right here, about 160 of these we were not able to categorize. There was not adequate data in the abstract or the published case report in order to select one fundamental underlying medical condition. You see that there is a wide variety of other conditions here. So in the next slide, I’m going to eliminate the 160 so we can expand these other conditions to show you the most common underlying conditions in these patients.

You see that hematologic conditions were the most common, neonates, cardiovascular, GI, solid tumors, and so on. I draw your attention to the color schemes here. Remember, red is fatalities and the blue is uninfected. So there are in some of these disease entities some patients who appear to be uninfected. But the other thing that is equally striking is that there are fatalities in a wide number of these different situations -- solid tumor, trauma, renal, transplant. With the transplant, we were a little surprised. These were all solid organ transplants. There are no hematopoietic stem cell transplants in here, which I kind of expected.

Point number one here is that the transfusion-transmitted disease occurred in a wide variety of patients, some of whom don't fit the traditional published risk categories.

Next let’s look at age, because that has also been widely touted as a risk category. Again, unfortunately, on the far right there are about 125 of these patients where age was not specified. So I’m going to move on so we can expand the data a little bit more.

Just to help you recall, category 4 is fatalities. You see that there was one fatality here in, not a neonate, but less than 1 year of age. The fatalities are strung out here. There’s not much in the way of young adults. But these fatalities are not all in old adults. Here is the 40-year range, the 50-year range. Category 1 is asymptomatic. This is symptomatic treated with antibiotics alone. Here is symptomatic with other complications. So again the disease is spread through a wide range of age of patients.

Now let’s look at the indications for transfusion. Again, unfortunately, a large number of these were not specified. So I’m going to go to the next slide and eliminate this so that we can spread out the other indications for transfusion.

On the left here is anemia of prematurity -- obviously these are neonates -- cardiovascular surgery, GI bleeding, GI surgery, hematologic, severe nosebleeds. Severe epistaxis resulted in death. Again, the red is the fatalities, and again you see they are fairly well distributed amongst a wide variety of indications for transfusion.

I want to draw your attention to the two columns over here. As soon as I say don’t look at this, you will look at it. So let’s focus over here. What we have broken down here is the kind of blood component involved in the transfusion. One hundred thirty-three out of 165 were red cells. There were also seven platelets, one specified as whole blood, one as apheresis, five not specified. There were no units of FFP and then 25 other cases in which the particular blood product was not specified.

This is another way to show the same thing. Almost all of these are red cells. The red here is not death. It’s infection. Here are the platelets. No units of FFP. Then in a large number of the cases, the blood products were not specified.

Here is another way to look at the outcomes in relation to the red cell units: uninfected, asymptomatic, uncomplicated, complicated, death, and unable to categorize. So there is a considerable breakdown in the severity of the transfusion-transmitted disease in these patients.

This shows red cell age in relation to these outcome categories. Recall that number 4 is fatalities. As you see here, some of these red cells are not stored very long, 10 days, 15 days. This is complications, uncomplicated disease, and asymptomatic disease. There are only 29 cases here, because again many of the reports didn't give either enough data for us to categorize the severity or they didn't give the age of the blood product transfused. So we are only able to get data for 29 cases.

This is something that Dr. Herwaldt referred to, the types of red cells or the nature of the red cell processing. Here is one unit that was collected by red cell apheresis that resulted in a fatality. A unit stored in Adsol, a unit stored in CPD. I'm not sure what the authors meant by "refrigerated." We usually refrigerate all of our blood, so it doesn't help a lot. But I call your attention again to the deaths, which is why Deanna circled this. Here's frozen/deglycerolized units, four of them. With one, the recipient was symptomatic, uncomplicated, symptomatic, complicated, fatality. Here is irradiated units, fatalities with irradiated units, also leukoreduced units.

So it isn't apparent that there is any particular processing method that reduces the risk of transfusion of disease.

Platelet units are a little difficult to identify. There do seem to have been some cases associated with platelets. One recipient was asymptomatic and one was symptomatic and complicated. In the previous slide I showed you, it appears that one of these may have been related to apheresis. Then for several in the published literature, we couldn't find much information about the nature of the platelets. I think the bias is, as you have heard from Dr. Herwaldt, that if the disease is transmitted, it's probably from whole blood-derived platelets that will have some red cell contamination remaining.

The conclusions that we have come to from these 65 literature reports are:

• First of all, no risk has been identified yet from fresh frozen plasma.

• Both red cells and platelets appear to transmit.

• Risk from red cells is far greater than from platelets.

• Red cells that have been frozen and deglycerolized, irradiated, or leukoreduced have all transmitted disease.

• Not clear whether apheresis platelets really are involved or not.

• Units seem to be infectious throughout their storage time.

• There were no deaths due to babesiosis in the 0 to 1 age group.

• All age ranges still reported complicated disease and deaths.

• GI surgery was the transfusion indication with the worst outcomes.

• GI disease is the medical history with the worst outcome.

This concludes the work that Deanna Fang did. She wants to thank Sue and wanted to thank me. But she did all the dirty work. She really was very compulsive in trying to find every single one of these case reports, and I applaud her for what she has done.

Thank you very much.

DR. JACKSON: Thank you very much, Dr. McCullough.

Our next speakers, before the break, will be Dr. Mikhail Menis and Dr. Richard Forshee, from OBE in FDA, on evaluating risk of *Babesia* infection in the US and the benefit-risk assessment for testing blood donations.

Agenda Item: Evaluating Risk of *Babesia* Infection in the United States

DR. MENIS: Our presentation is on evaluation of risk of *Babesia* infection in the United States using CMS and CDC data.

The goal of our evaluation was to establish a database to support benefit-risk analysis for *Babesia* donor testing. The objectives were to assess babesiosis occurrence among the elderly in the United States using CMS databases, as well as to evaluate babesiosis occurrence in the general population using CDC data, and then to compare babesiosis occurrence overall and by states using CMS and CDC data.

The key point of this analysis is that it substantiates the use of CMS databases for the benefit-risk analysis of *Babesia* donor screening strategies.

We utilized CMS databases and CDC data to assess occurrence of babesiosis in the United States. Specifically, we used CMS administrative data for calendar years 2006 through 2013 to ascertain incident babesiosis cases, based on the first recording of babesiosis, without prior history of babesiosis diagnosis in the preceding year. Babesiosis occurrence rates per 100,000 US elderly were ascertained overall and by calendar year, diagnosis month, and state of residence. We also used CDC data for 2011 through 2013 to assess babesiosis occurrence rates per 100,000 residents utilizing US Census data. Then we compared ranking of states for CDC and CMS data, based on babesiosis rates.

The next couple of slides will be on CMS data results. Overall, during the eight-year period, CMS data investigation identified 10,301 unique US elderly Medicare beneficiaries with a recorded babesiosis diagnosis and a national babesiosis rate of about 5 per 100,000 elderly Medicare beneficiaries, with state-specific rates up to 10 times higher than the national rate, with significantly increasing babesiosis occurrence in the United States during the eight-year period and the highest rate in 2013, again with the highest babesiosis rates in June, July, and August. Seventy-nine percent of all cases were diagnosed from April through October, similar to the CDC results.

This figure shows babesiosis occurrence among the US elderly by county of residence. As you can see, virtually all the states nationwide except for Wyoming had babesiosis recorded in the elderly, with a substantial concentration of cases occurring in the Northeast corridor, specifically in the top five endemic states.

You can see here in this figure babesiosis cases, the gray bars, and rates, black line, by year among the US elderly Medicare beneficiaries during the eight-year period. As you can see, from 2006 through 2013, babesiosis occurrence is increasing, with the largest number of cases and rate in 2013.

This figure shows babesiosis cases and rates by month of diagnosis among the US elderly Medicare beneficiaries during the eight-year period, with the largest number of cases in June, July, and August, and the smallest number of cases in January, February, and March. Again, trends are similar to the CDC data.

Table 1 shows babesiosis cases and rates among the US elderly Medicare beneficiaries overall, by state and year, during the eight-year period. The states are sorted in descending order of babesiosis rate, with the highest rates occurring in the states of Connecticut, Massachusetts, Rhode Island, New York, and New Jersey. Other highlighted states are *Babesia*-endemic states of New Hampshire, Maine, Minnesota, and Wisconsin.

This slide highlights overall babesiosis cases and rates for the top 15 states, sorted by babesiosis rate on the left in Table 1a and by number of babesiosis cases in Table 1b. As you can see, whether sorted by babesiosis rate or babesiosis cases, the same states end up in both tables, with the highest rates in Connecticut, Massachusetts, Rhode Island, New York, and New Jersey, with the rates per 100,000 in parentheses.

These top five states -- Connecticut, Massachusetts, Rhode Island, New York, and New Jersey -- accounted for 76.6 percent of all cases identified in the US elderly. The nine endemic states, including the top five states plus Minnesota, Wisconsin, New Hampshire, and Maine, accounted for 80.2 percent of all cases in elderly. Other states, as you saw, also had babesiosis recorded, including but not limited to Maryland, Virginia, Pennsylvania, Florida, and California. The top 15 states, from Connecticut to Florida, by descending babesiosis rate, accounted for 92.6 percent of all cases. The majority of those 15 states also have significant trends over time.

The next slides will be about presenting CDC data on babesiosis occurrence. This Figure 4 shows state-level distribution of babesiosis cases as reported to CDC during 2011 through 2013. As you can see, babesiosis was not reportable in 19 states during the whole period, those states highlighted in gray. They include Pennsylvania, Virginia, and Florida, which do have a substantial number of cases based on CMS data.

Figure 5 shows number of reported cases by year using CDC 2011 through 2013 data, with the highest number of cases occurring in 2013, similar to CMS data. Figure 6 shows number of babesiosis cases by month of symptom onset, CDC 2013 data. Similarly, the largest number of cases occurred in June, July, and August.

This Table 2 shows babesiosis cases and rates in reporting states overall and by year using CDC 2011 through 2013 data, with the states sorted in descending order of babesiosis rate, with the highest rates highlighted in yellow. They are the nine endemic states, starting from Rhode Island, Connecticut, Massachusetts, New York, New Jersey, Maine, New Hampshire, and Wisconsin. Again, the ranking is similar to CMS data, based on babesiosis rate.

The next slide pretty much highlights Table 2, with overall babesiosis cases and rates for the top 15 states, sorted by babesiosis rate on the left and sorted by number of babesiosis cases on the right. As you can see, the top nine states are the same, the endemic states of Rhode Island, Connecticut, Massachusetts, New York, New Jersey, Maine, New Hampshire, Wisconsin, and Minnesota.

In summary, the highest overall babesiosis occurrence rates using CDC data also occurred in the five Northeastern states of Rhode Island, Connecticut, Massachusetts, New York, and New Jersey. These top five *Babesia*-endemic states accounted for 85.2 percent of all cases reported to CDC during 2011 through 2013. The top nine endemic states, including the top five states plus Minnesota, Wisconsin, New Hampshire, and Maine, accounted for 98.5 percent of all cases reported to CDC. The top 15 states from Rhode Island through Nebraska, by descending babesiosis rate, accounted for 99.4 percent of all babesiosis cases reported to CDC.

This slide shows a comparison of CMS and CDC estimates of babesiosis rates and corresponding rankings for the top 15 states. As you can see, rankings are similar, especially for the top *Babesia*-endemic states. For example, if you take Connecticut, it was ranked number 1 based on CMS data and ranked number 2 based on CDC data. New York was ranked number 4 based on CMS data and number 4 based on CDC data. The same ranking occurred in New Jersey and in New Hampshire, which was ranked number 7 in both. So you can see that the ranking based on babesiosis rate is similar for CMS and CDC data.

However, there is a substantially higher rate of babesiosis occurrence using CMS data as compared to CDC data, almost an order of 5. The next slide will try to summarize that.

Overall, babesiosis results on rankings of state and on occurrence trends over time and by diagnosis months were similar for CMS and CDC data. However, babesiosis occurrence rates identified using CDC case reporting data in general population were substantially lower as compared to babesiosis occurrence identified by CMS data in the US elderly, which could be due to underreporting or lack of reporting to CDC and a higher likelihood of under-diagnosing babesiosis in the general population versus elderly since babesiosis is more likely to be asymptomatic in younger individuals as compared to older persons. Therefore, we believe that babesiosis occurrence rates among the US elderly Medicare beneficiaries based on CMS data provide the best available population-based estimate of babesiosis occurrence in US blood donors. As such, it was further used to assess number of TTB units prevented and false-positive units diverted, overall and by state, for different blood donor testing strategies, as will be presented next by Dr. Forshee.

CMS data do have limitations. They include:

• Difficulty in identifying incident versus prevalent cases.

• Possible misdiagnosis or mis-recording babesiosis diagnosis.

• Lack of clinical detail for diagnosis code verification.

• Lack of clinical information to ascertain *Babesia* species.

Thank you so much. I would like to acknowledge the following FDA and CMS and Acumen participants. Now Dr. Forshee will present.

Agenda Item: Benefit-Risk Assessment for Testing Blood Donations for *B. microti*

DR. FORSHEE: Good morning, everyone. I'm Rich Forshee, with the Office of Biostatistics and Epidemiology.

Today I'm going to be presenting the results from the benefit-risk assessment that we conducted to better understand the implications of using serology or NAT in different selections of states in terms of testing for babesiosis.

The primary outputs that we are looking at in the model are, probably most importantly, the units from donors with babesiosis that are identified and interdicted and the percent of risk reduction from the status quo. The risk reduction was calculated by dividing the units interdicted divided by baseline units from donors with babesiosis that are occurring under the status quo with no testing.

In addition, we calculated the positive predictive value. Just as a reminder, the positive predictive value is the number of true positive units divided by the number of true positive plus false-positive units, and we estimated the number of donors who receive a false-positive test result. A note on this last: We are referring to donors, not donations, as we are in the first bullet point.

The primary data inputs for the model were the state-level babesiosis rates from CMS and state population for residents 16 years and older, which were taken from the US Census. Dr. Menis has already described how the state-level babesiosis rates were derived from CMS, as well as some of their limitations.

In addition, we had to make a number of assumptions in order to estimate the outputs that we cared about. I have listed the key assumptions here. We assumed that about 5 percent of the population 16 years and older donate blood, that the average donor donates 1.7 times a year. We do not have an approved serology or NAT test, so we had to make assumptions about what that performance might look like. We assumed a 97 percent sensitivity for a hypothetical serology and a 99.98 percent hypothetical sensitivity for serology and NAT. We also had to make some assumptions about how long it would be before each test could detect the parasite or antibodies. Our baseline assumptions are that it would be about 14 days before NAT can detect the parasites in a blood sample and 21 days before antibodies are detectable.

We further assumed that the tests would have a very high specificity of 99.98 percent. We assumed this specificity would be the same whether you were using serology only or serology plus NAT.

We will talk a little bit about some sensitivity analyses that we did in terms of what would happen if we deviated from some of these assumptions that we made.

I want to emphasize that there are some major uncertainties in the modeling that I am going to be presenting. As I already mentioned, we don't have an approved donor screening test for babesiosis, so we don't have good data on what the sensitivity or specificity of an approved might be. Dr. Menis has already discussed the limitations of the CMS data that we are using. Again, while there are limitations there, we think that overall they are the strongest data for us to use for this analysis.

In addition, we don't know much about the progression of babesiosis in humans. This is relevant to the question of how long it’s going to be before NAT would be able to detect the parasites. We also don't know the probability of infection for a patient that receives a unit from a positive donor.

We did conduct several sensitivity analyses to explore how the results would change using alternative values for inputs, such as shorter or longer window period before NAT can detect the parasites. I will describe a few of these at the end of the presentation.

The final point that I want to make before I get into the results themselves is that we believe that the percentage risk reduction and the positive predictive value outputs are likely to be more robust than the outputs of the absolute numbers of donations or units. The reason for this is that both the percentage risk reduction and the PPV are ratio variables that include some of the uncertain variables in both the numerator and the denominator. For example, if we are off about what the babesiosis rate is, some of that would cancel out in terms of what would be in the numerator and the denominator. Nevertheless, we think the absolute numbers are useful in providing some sense of scale, but they are sensitive to the assumptions that we have made.

I want to start by showing this double-dot plot that includes all of the states of the US. I'm going to zoom in on the states of most interest in the next slide. There are a couple of things that I want to mention about this slide. Let me start to describe it. I'm showing two series of data on this slide. The first series of data shows the number of positive units that are detected and the second shows the percent risk reduction. I have sorted the states here on the basis of their state-level babesiosis rate as estimated from CMS, starting with Connecticut, with the highest rate, going all the way down to Wyoming, which didn't have any detected babesiosis in the CMS data.

The first thing that I want you to note about the chart is that you do start capturing a lot of risk reduction in the first few states that have the highest babesiosis rate. There is a very quick opportunity to get a major risk reduction in some of those highest babesiosis rates. The marginal risk reduction does begin to slow as you include more and more states that have lower babesiosis rates, but there is not a completely obvious choice about where that line should be drawn based on the risk reduction.

A couple of other things to mention. New York contributes the largest number of positive donors. This is a reflection of the fact that it both has a high babesiosis rate and a large population. It is the single largest contributor of positive donors and risk reduction in the model that we have constructed.

The other point that I would like to note is that once you get to around the New Mexico level, many of the remaining states, our model suggests, are contributing somewhere between zero and two positive units. So there are a large number of states where there are a small number of donors that are predicted based on our model.

The next point that I want to mention is a zoomed-in version of that same chart. This is the same chart design. We are just stopping now after California. The first thing that I want to note is that a serology-only strategy targeting the states with the five highest babesiosis rates would reduce TTB risk by about 70 percent. We can capture 70 percent with the first five states using serology only. If we expand this out to include a total of 15 states plus the District of Columbia, going all the way down to capturing Florida, we now get up to about an 84 percent risk reduction using those top 15 states plus DC.

I want to point out one difference between the collection of states that we are talking about here and those that were included in the issue summary. In the issue summary we went to California, including that as the 16th state. After some further internal discussion, we decided to remove California from this because of the different species that is predominant there. To avoid any confusion between what I am presenting today and what you may have seen in the issue summary, this is the difference. We are not including California in the 15-state-plus-DC strategy.

Again, this is assuming our baseline assumption of 97 percent sensitivity and a 21-day window period for serology.

Up until now, I have been showing this sorted by the state-level babesiosis rate. Another reasonable way to look at the data is to sort it by the predicted number of positive donors that you may see in the state. This is the same type of chart, with positive units on the left, risk reduction on the right, but now we are sorting it by the number of positive donors from the state. So the data are the same, just ordered differently. New York still contributes the largest number of positive donors. New York by itself provides almost a 32 percent risk reduction from baseline.

One thing that does change is that some of the large-population states suddenly jump very high on the list. So California and Florida would become number 5 and 6 if we were sorting this by the number of positive donors that were expected.

A couple of states do drop out. I apologize, we should strike Rhode Island out of this bullet point. Rhode Island does appear in both lists, but Delaware, DC, and Vermont are not on the list when you sort it by the number of positive donors. Again, please strike Rhode Island out of that bullet point.

Next I want to give you some idea about what happens with positive predictive value as we choose different sets of states for inclusion in a testing program. Again we are going to look at serology-only testing here to give you an idea of what that looks like. If you limit your testing to only the highest states, such as the top three states here of Connecticut, Massachusetts, and Rhode Island, your positive predictive value is up around 69 percent, which is very reasonable for a screening program. As you begin adding more states that have lower babesiosis rates, as expected, the positive predictive value begins to fall. If you include all 50 states with serology, the positive predictive value will fall to 19 percent.

One thing that I want to mention here is that because of the assumptions in our model that you have the same specificity whether you are using serology only or serology plus NAT, the positive predictive value actually increases a little bit when you start adding NAT to additional states. The reason for this is that the false positives are going to remain the same, but you are going to be picking up a few more true positive units because of the shorter window period and the slightly greater sensitivity.

Next I want to look at the additional risk reduction that we gain when we begin using NAT in addition to serology. As I just mentioned, the reason that NAT is going to provide some additional risk reduction is that it shortens the window period when the infection can’t be detected and it also improves the sensitivity.

If you added NAT to all states -- so if you have serology plus NAT in all states -- the NAT will yield an additional 4.7 percentage points of risk reduction. Most of that additional risk reduction comes in the first set of states, whether you would look at the first five or so or if you would go all the way down to Florida. Going to Florida increases the risk reduction by 4.3 percentage points.

Those charts were to give you an idea of what this looks like on a continuous level, looking across all the possibilities of what states may be included. We have had some internal discussions, and we are going to present results for some selected testing strategies to reduce transfusion-transmitted babesiosis that we think are candidates for striking a good balance between risk reduction and limiting the number of false-positive donors that are detected. The committee should not consider these to be restrictive of your considerations, but we thought that they were good candidates to allow you to have your deliberations.

This is a quick overview of the strategies that we are going to be looking at. We are moving from the least extensive testing, which would be serology only in just the top five states, all the way to a maximum that would look at serology plus NAT in all states plus the District of Columbia. I want to go through these in a little bit more detail and then show the summary results. You have the summary results at your table as well.

The minimum results focus on the classic five endemic states. We can have serology only or serology plus NAT. We are going to follow a consistent color scheme as we go through these charts.

Expanding out to nine states, I should mention that these nine states were not based on any single data source. We did not simply look at the CMS data or the CDC data. We considered both CMS and CDC data, as well as information on where the diseases were endemic and where we have seen transfusion-transmitted cases, in order to get what we thought was a reasonable set of nine states for this next strategy.

We also looked at a 15-state-plus-the-District-of-Columbia strategy, which includes this entire set of states, which you can see on the map. We have listed the two-letter state abbreviations.

Another one that we included was serology in 15 plus NAT focused in the five highest endemic states. The final set begins with serology in all 50 states and gradually adds NAT to either the five highest, the set of nine that we believe have endemic and transfusion transmission, the set of 15 plus the District of Columbia, and, finally, serology plus NAT in all 50 states plus the District.

You can probably see this next summary table better on the handout that is included at your table. What we have tried to do here is to pull together all of the information in a single graphic. For those of you who prefer tables, there is an updated Table 2 that is included in your packet as well that has the same information, so whichever is your preferred way of sorting through the information. Let me walk through what we are showing in this chart to make it a little easier to digest.

Across the top we are showing the 12 selected testing strategies that we are presenting results for here. These are ranging from the least amount of testing to the most extensive amount of testing. We have four rows of drop-line plots that are indicating the major model outputs. The top row is the TTB risk reduction in percentage. The next row is the positive units interdicted, followed by positive predictive value and donors with false-positive results. As you read across, you can see the estimated value for each of those, as well as the trends that we see as we are adding more states.

I want to start by discussing the trends in the TTB risk reduction. The first thing that I would like to draw your attention is that if we are looking to get something on the order of a 90 percent or 1-log reduction in risk, we are going to need to go out to probably a 16-state strategy that includes some NAT testing. If we move to serology in 50 states, we are already going to be capturing a 91 percent risk reduction. Moving to serology in all 50 states plus DC, adding NAT in the five highest babesiosis-rate states, we move up to 95 percent risk reduction. After that, adding NAT in additional states does have some additional risk reduction, but it is oftentimes less than 1 percent for each additional scenario that you look at.

You can track the exact numbers of positive units interdicted. For example, going from serology in 50 states, NAT in five, you are at 975 positive units interdicted at that point. Going out to the nine-state strategy, you get an additional three units that would be predicted to be interdicted.

Next looking at positive predicted value, you can see that positive predictive value in the five-state strategy starts out at 57 percent for positive predictive value. Adding NAT, as we discussed, bumps up positive predictive value a little bit by detecting a few more cases. As you expand out serology or serology plus NAT, the positive predictive value drops. At 15 states plus DC, we are around 39 or 40 percent, depending on how extensive the NAT testing is. Going to serology in all 50 states drops us to 19 percent, and we stay at that same level regardless of how much NAT testing is being done.

You see a similar story when you are looking at donors with false-positive results, which steps up as you add on an additional level of states. So we have the classic tradeoff between the risk reduction that you are going to have and the number of donors that you may lose due to false-positive test results.

We want to make a point of emphasizing the need for very high specificity for any of the tests that would be used for a screening program like this. The assumption that we were using was a specificity of 99.98 percent. You saw the positive predictive values that we were getting under that scenario. This graph shows the specificity along the horizontal axis and the positive predictive value on the vertical axis. As you can clearly see, even small reductions in the specificity of the test are going to quickly lead to a much lower positive predictive value. Of course, the reason for this is that the test is going to be applied to millions of people who are not infected with *Babesia*, and therefore even small decreases in specificity will lead to a lot of donors being identified as false-positive.

As I mentioned, there are a number of assumptions that we had to use in this model. The results would change if some of those assumptions turn out not to match reality. As I mentioned in the earlier slides, we do believe that the ratio variables of TTB risk reduction and positive predictive value are going to be less affected by changes in these assumptions than the counts that we showed. The results could also be affected by the estimates of the babesiosis rate and blood donation rate.

I want to show you a couple of the sensitivity analyses that we conducted. This is not the full set that we conducted internally, but they give you a flavor of what the magnitude is. One of the potential sources of uncertainty is how long it will take before either serology or NAT would be able to detect the infection. Under the baseline assumptions that I showed, if we were doing serology in all 50 states plus NAT in five states, we have about a 95 percent risk reduction. If you assume instead that it takes 21 days instead of 14 days for NAT to detect the parasite and 28 days versus 21 days before seroconversion, that 95 percent risk reduction drops to a 93 percent risk reduction. Remember, we had assumed a sensitivity of 97 percent for serology. If the serology turned out to be lower for a test, if it came in at 95 percent, the risk reduction would drop from 95 percent to 94 percent.

So there would be changes, but these are not huge changes in the percent of risk reduction. It is also important to note that it doesn’t change the relative value of the states that are included.

Under the baseline assumptions of positive predictive value, we had a 19 percent PPV. If the specificity falls to 99.8 percent from the 99.98 percent that we assumed, the positive predictive value will fall to 2 percent because of the number of additional false-positive donors that would be detected.

A couple of concluding points. The specificity of both NAT and serology needs to be very high in order to minimize the number of false-positive donors detected and maintain positive predictive value. Assuming that we started with nationwide serology -- and that is just an assumption for getting an idea of what the magnitude of NAT would be -- nationwide NAT plus nationwide serology would reduce risk by an additional 4.7 percentage points above what you would achieve with serology across the nation.

The other point that I want to make is that there are several possible testing scenarios that have similar benefit-risk profiles. This is a situation where I don't think the benefit-risk results by themselves are going to point to the absolute best answer. I think that considerations about risk tolerance and what tradeoffs we are willing to accept are going to have to be part of the discussion to come to a considered decision regarding these issues.

With that, I will just say thank you very much. It’s an honor to have the opportunity to share my results with you today.

DR. JACKSON: Thank you very much.

We will go ahead and take questions at this point.

Agenda Item: Questions for Speakers

DR. MAGUIRE: I have been impressed, watching the history of babesiosis since the 1960s, by its spread. It started out in a few offshore islands and now it is in many states and it’s still spreading. I guess this would be a question for Dr. Herwaldt. Do we have information on where it is spreading now? Where is it going to go? Are there any models? I think this is not a static situation. This is a very dynamic situation. If you look at the distribution of Lyme disease, which is much, much broader than that of babesiosis, I suspect that *Babesia* distribution will probably fill in the empty spots in the Lyme distribution.

DR. FORSHEE: I think the one thing that I can say with regard to that goes to the results that were presented by Dr. Menis. In CMS we were seeing a statistically significant upward trend in the babesiosis rates that were reported in CMS.

I don't know if Dr. Herwaldt would care to comment on this.

DR. HERWALDT: Of course, Jamie raises a very good point. It’s a complex issue that I am not fully prepared to address, in the sense that I’m not a modeler. But I agree with Jamie that over time there are foci and states, so to speak, that have been added to the map. I’m not saying this next point for the purposes of regulatory issues per se, but Pennsylvania is an example of a state in which, certainly in some of the eastern parts of the state, it is well-established now. I talked with the Department of Health this past week to confirm that. There was a paper that just came out in an entomology journal that looked at tick infection rates. But also clinical data and epidemiologic data indicate that there are foci of endemicity now in eastern Pennsylvania.

Pennsylvania is not on our list of states in which it is a reportable condition, not because of lack of interest there, but, as Al DeMaria knows very well, in certain states it can take quite a bit to make something reportable -- laws and regulations, et cetera.

But in terms of Jamie’s big-picture question, in terms of tick infection rates and ecologic changes and all of that, in terms of how foci may be expanding and increasing, of course, it gets into complexities.

The analogy with Lyme is important, but also, of course, imperfect, as Jamie knows. How did Andy Spielman refer to it? Lyme travels on --

PARTICIPANT: -- birds and *Babesia* travels on --

DR. HERWALDT: The backs of mice. Thank you, Susan. That’s exactly right.

For those of you who didn’t hear that, Andy Spielman, who was an expert -- he is deceased now, unfortunately -- an expert entomologist, spoke about Lyme, or *Borrelia burgdorferi* spreading on the backs of birds and *Babesia microti* on the backs of mice.

For whatever it’s worth, the surveillance data for Lyme is about 30,000-some cases per year. Of course, there are issues with what constitutes Lyme disease and case definitions, et cetera.

I don't want to go on and on. Jamie, do you want to chime in?

DR. BAKER: Is there any data on race/ethnicity in both of the donors and the clinical cases, and mortality?

DR. FORSHEE: With regard to the modeling that we did, we did not include any race/ethnicity in terms of the modeling exercise that we did.

DR. BAKER: Thank you. Also for the other speakers, Dr. McCullough or Dr. Herwaldt?

DR. MCCULLOUGH: I’m trying to remember the statistics. There were a number of cases in sickle cell disease patients. They are in the hematology category. I think there were at least three or four deaths. There is no good denominator, so it’s hard to know what to make of that. But hematology was the number-one category of patients who became infected, and they had, I think, the second-largest number of deaths.

DR. LERNER: With regard to that, just out of curiosity, what proportion of those hematology cases were, in fact, sickle cell disease? Would you know? It’s just broadly called “hematology.”

DR. MCCULLOUGH: You are recording this, so I would hate to give a number. But I think it was about half, actually.

DR. LERNER: Thanks so much.

DR. BAKER: Secondly, in the mortality due to the excessive nosebleeds, the epistaxis, was there any indication of underlying von Willebrand disease?

DR. MCCULLOUGH: I think that was only one patient. The indication for transfusion was epistaxis, and the patient died. Obviously the patient didn’t bleed out from epistaxis. As I recall, I don't remember the underlying disease in that patient, but they died of the underlying disease, which we counted as a death. But the reason for the transfusion was very severe epistaxis.

DR. BASAVARAJU: I just have one question. I’m not sure which speaker would answer it. Maybe it was presented, but I didn’t see it. Do you know what proportion of people who have been infected with *Babesia* develop chronic infection? What is the longest documented period of chronic infection that we know of in somebody who has had *Babesia*?

DR. KUMAR: I think the data is very limited in terms of how many infections result in asymptomatic, silent infection. We have no active case detection studies. Most is passive. Recall one study with Peter Krause, where he did find that 50 percent of children and 25 percent of adults remain asymptomatic. But these again were passive detection, not active case detections.

What was the second question?

DR. BASAVARAJU: What is the longest period of chronic infection?

DR. KUMAR: The longest period again is in Peter Krause’s study. In one patient in Connecticut, western Connecticut, he found at 27 months PCR positivity. But this is just one detected. It’s not to say that -- it may last longer. But we don't know in what proportion of infections it lasts that long. The majority of them, as the data is emerging now, do clear infection by three to five months, became non-detectable by PCR.

DR. LEITMAN: Could I comment on that? There are also case reports of documented treatment that was unsuccessful with persistent high serology and persistent intermittent PCI. There is one case that is at least 24 months. They are rare, but they happen.

DR. KUMAR: That is correct, yes. Thank you.

DR. BAKER: This is for Dr. Menis. Your Figure 1 map of babesiosis occurrence, was that *microti* or all types?

DR. MENIS: CMS does not distinguish between specific species. We assume that if it is a *Babesia*-endemic state, it would be *Babesia microti*. But it’s one of the limitations that I mentioned during the presentation.

DR. JACKSON: We are now going to take a 15-minute break. We will be back here about 10:20.

(Brief recess)

DR. JACKSON: If we could have Dr. Susan Stramer, from the American Red Cross, for presenting results on “Investigational Blood Donor Screening for *Babesia microti*: Implications for Blood Safety.”

Agenda Item: Experiences with Investigational Testing of Blood Donors for *B. microti*

Investigational Blood Donor Screening of *Babesia microti*: Implications for Blood Safety

DR. STRAMER: It’s a pity that people aren’t back from break yet.

DR. JACKSON: Well, you said you are going to be over your time.

DR. STRAMER: Yes, vast quantities of data.

While people are streaming in, I will thank the FDA for the opportunity to present our data, investigational data, that has been collected in collaboration with our partner, IMUGEN, who is the sponsor for the investigational study.

The title of my talk, just to consume time while people float in, is “Investigational Blood Donor Screening for *B. microti*: Implications for Blood Safety.”

I would like to acknowledge my collaborators, both at the American Red Cross and at IMUGEN.

The early part of my talk will be a review. *B. microti*, as we know, is an intraerythrocytic tick-borne parasite. I'm showing you the obligatory tick on a coin, as most *Babesia* talks include. Babesiosis is the resulting disease that ranges from asymptomatic to fatal.

Transfusion-transmitted babesiosis has already been reviewed in Dr. Herwaldt's case series of 159 well-documented cases of *B. microti* responsible for TTB between 1979 and 2009 in the United States. As was already highlighted, 87 percent of those occurred in seven endemic US states, 77 percent between the years of 2000 and 2009, the last decade represented. Three additional cases occurred from *B. duncani*, which occurs on the West Coast. The three cases were two in California, one in Oregon. There have been four cases in random donor platelets; otherwise, none reported in this series from apheresis platelets. But all others were in components that were red blood cells. It resulted in 28, or 19 percent, fatalities.

Again, there is no licensed blood donation screening test for this agent.

Babesiosis, as we heard, is a malaria-like illness with general mortality from 6 to 9 percent, 21 percent generally reported in immunocompromised individuals. Dr. McCullough went through the recognized risk groups and did point out that most cases do not occur in any risk group that is traditionally reported as *Babesia* at risk. I will show one slide to reiterate that. It can occur in any recipient group, anyone who receives a blood component.

Symptom onset is reported as variable, but generally one to nine weeks following a tick bite or a transfused positive unit. Symptoms can be nonspecific and result in hemolytic anemia. As Barbara already highlighted, the hallmark of a diagnostic blood smear for *B. microti* is the tetrad, or the Maltese cross.

Just to summarize what Jeff McCullough presented earlier this morning, from the Herwaldt series, this is discrimination of cases by underlying condition and the transfusion indication. Those in red are the traditional *Babesia* at-risk groups -- hematologic, trauma, or neonates. You add those all together and they do add to less than 50 percent. Again, to reiterate Jeff's point, babesiosis can occur in any transfused recipient.

This we have seen before. Also there are many species of *Babesia* that are present in the United States. The one that we are most concerned about is *B. microti*. It's the majority of the problem. It primarily occurs in seven endemic states, which are indicated in turquoise, as listed on the slide. Other species of *Babesia*, with the exception of *B. duncani*, have never been involved in a transfusion-transmitted case.

Looking at the CDC data, as has been shown, we know it became nationally notifiable in 2011, with cases increasing and number of states reporting increasing through 2013, with 1,762 cases reported in 2013.

Looking at this on a map and reiterating what the CDC published on their website, 95 percent of the 1,762 babesiosis cases reported in 2013 came from seven states. So I would say that we have a regionally limited problem to conquer.

Looking at what the FDA posted on their issue summary page, again you can see the seven states that are highlighted in dark blue. I will talk to you about those seven states plus two more states, which include New Hampshire and Maine, where we have seen an increasing number of transfusion-transmitted babesiosis cases.

Here are the nine states that I will consider and you will hear from other statements are those that are most relevant to the discussion today: Rhode Island, Connecticut, Massachusetts, New York, New Jersey, Maine, New Hampshire, Wisconsin, and Minnesota. You can see on my right the column that is babesiosis rate per 100,000. You can see the rates that are reported in the CDC reporting. Everything other than those nine states reports less than 1 in 100,000 -- actually, less than .5 per 100,000. You are going to hear me use a rate of 1 in 100,000 kind of as a trigger not only for statewide reported cases of *Babesia*, but for TTB cases of *Babesia*. That is a trigger that we can use as a measure of when screening should expand.

Jamie asked earlier about the extrapolation of Lyme disease relative to *Babesia*. We know that Lyme does travel more quickly or more rapidly, extensively, than *Babesia*, because it can travel in birds. Certain birds are reservoir-competent, and they can transmit Lyme. So even though we may be talking about nine states, we know that we need to be vigilant, since expansion will occur. This is a very dynamic situation, as shown on this slide of 17-year trends for Lyme disease.

The American Association of Blood Banks, AABB, last summer issued an Association Bulletin covering babesiosis and TTB. We first talked about potential interventions that can be used to combat TTB. We currently ask a donor screening question about a history of babesiosis. Obviously, if that was effective, we wouldn’t be here today. It’s unlikely to be accurate due to poor donor recall. Most cases of babesiosis are asymptomatic or mild in healthy individuals -- that is, in blood donors.

We can ask donors about tick exposure, which would be highly nonspecific and does not have the ability to differentiate seropositive donors versus control donors, as has been published. Infected patients often do not recall tick bites, because the ticks that carry *Babesia* and are dependent on a blood meal for growth are the nymph stage and they are extremely tiny. Donors reporting tick bites are less likely to be infected, because they are more vigilant and they remove the ticks promptly, within the 24- to 48-hour grace period.

The last option we are left with is testing by antibody and for DNA.

Just to show you the size of a tick -- this will probably cure you from eating a poppy seed bagel -- as we see here, the tick that gives you, in this case, Lyme disease and *Babesia*, the same tick, is smaller than a poppy seed. I would also venture to say that in Florida there is probably as great a risk from elderly Jewish people on Collins Avenue in Miami Beach eating a poppy seed bagel that may contain a nymphal stage of this, as was shown to you in the CMS modeling data.

So what did we recommend? The AABB recommended that hospitals and blood centers in *Babesia*-endemic areas consider what interventions are available and may be appropriate to reduce the risk of TTB. Hospitals and blood centers interested in testing should contact an IND sponsor. Hospitals and blood centers should fully investigate and report to the blood centers all cases of TTB.

What do we do at the Red Cross? We have partnered with our sponsor, IMUGEN, and we are involved in an IND study. It's a screening protocol. We have been doing prospective screening since June 2012. We use an automated IFA, so it's not really an IFA. It's an arrayed fluorescence immunoassay. It's automated just as any screening tests we use in blood centers are automated. It has a throughput of 3,000 samples in three hours. With one setup, six runs a day, six days a week, you can do 100,000 samples in a week. It has the same throughput as any of our screening instruments today. It uses native *B. microti* antigens with a dynamic range of -- an endpoint of 128 through or equal to 1,024.

We also test with PCR, the DNA detection targeting the *B. microti* 18S ribosomal RNA gene, with a 95 percent lower limit of detection of 66 piroplasms per ml.

It is very important that we also do confirmatory testing. These are screening tests. We confirm by Western blot containing either IgM or IgG conjugates. We do an enhanced version of PCR that has more sensitive detection. We do quantitative PCR to get parasite loads. We do animal inoculation challenges.

We originally in this program targeted selected counties within endemic states for collections on certain days. We also target whole blood and red cell pheresis collections, because, as you have heard before, there are very few, if any, cases through platelets, none clearly documented through apheresis platelets, and pink platelets, or those produced by random donor collections, that have had red cell contamination. Using today's technologies, red cell contamination is very limited, even in a random donor platelet.

Reactive units are removed from the blood supply. Donors are deferred indefinitely and invited to participate in follow-up studies. We also contact hospitals and do look-back, which is from positive donors. Prior donations for a one-year period of time are retrieved and recipients are asked to be tested.

We did a retrospective study as part of the IND study, as requested by FDA. We used three different regions. The endemicity of the region is indicated by the increasing pinkness of the slide. We low-endemic areas, including Arizona and Oklahoma, moderately endemic, Minnesota and Wisconsin, and highly endemic, Connecticut and Massachusetts. We screened a total of 13,000-plus donations. The next columns show you the number of positives broken down by either PCR positivity, antibody positivity, or both.

The first column: In this study, we saw no window-period donations. We did find PCR-positives, two from Minnesota and Wisconsin and five from Connecticut and Massachusetts. We saw one true positive, antibody-positive, at a low cutoff, which I will talk about, from Arizona, three from Minnesota, and 33 from Connecticut. The prevalence figures are given to you in red -- .025 percent in the low-risk areas, .1 percent in the moderate areas, and .75 percent in the highly endemic areas.

This translated to a specificity, if we used a diagnostic cutoff, which we used for this retrospective study, of 1 in 64 at 99.5 percent, but at the screening cutoff, at 1 to 128, there was a 99.98 percent specificity. That includes both tests, antibody and PCR. I will let you know that that is better specificity than we see for any donor screening test that we use today for any marker.

This one antibody-positive donor was positive at a titer of 1 to 64. If we would have used our screening cutoff, we would not have detected this individual with remote prior infection. The Western blot shows you an IgG and an IgM conjugate Western blot, with positive and negative controls listed and then the donor sample. The 007GV is the donor sample. You can see that using the IgG conjugate, we did see bands. Using the IgM conjugate, there was nothing.

Let me read from the caption: The donor’s reactivity nature is unclear. He lived in New York for a period of time, spent time in Pennsylvania, but did not recall any time in wooded or grassy areas or finding a tick on his body. He was stationed in Germany from 1975 to 1976, where he reported tick bites. He does have dogs. He does have lots of outdoor activities. The bottom line is, this is a remote infection, and the nature of the infection is unknown.

Looking at the 13,269 donations that we tested, the zip code of donor residence is plotted here in the lavender. So even though we don't have a national prevalence study, this can show you in the six states that we tested for where the donors actually resided. If we consider that the one positive from Arizona would have not been detected or excluded based on a 1-to-128 cutoff, the only locations we saw positives were in the states of Minnesota, Wisconsin, Connecticut, and Massachusetts -- of course, because that is where we targeted screening. No other states had positive donors.

Now focusing on Massachusetts and Connecticut, we screened in the entire state of Massachusetts and only two highly endemic states [sic] in Connecticut. The yellow dots show you antibody-positives. Those dots with red stars show you the five PCR-positives we had in these two states. Of course, we had another PCR-positive in Minnesota that I am not showing you.

What we learned from this study is that, for prospective screening where I narrowed the focus of where we did screening, we only used the coastal areas. We selected ten counties on the coast of Massachusetts and Connecticut. Boston and Hartford were excluded because we needed sites -- this is an investigational study -- where donors could go to opt out of research who did not want to participate in the research.

What are our results for the calendar years of 2012, 2013, and 2014? This takes you through September 30 of 2014. We found 339 reactive of 90,000 donations screened, for a rate of about .4 percent. We found nine window-period units -- these were the NAT units that the FDA spoke about -- the rate being about 1 in 10,000. Let me put this in perspective for you. This is a 100-fold higher window-period yield than we see for HIV or HBV and 20-fold higher than HCV. I don't see any discussion of us talking about eliminating NAT for any of those three agents.

The breakdown by state: Again, for Minnesota and Wisconsin, it was about .1 percent, Massachusetts .2 percent, Connecticut .64 percent.

I’m going to show you a map of Massachusetts again and then Connecticut. Green are antibody-positives.

Orange and red are PCR-positives, orange being PCR- and antibody-positive and red representing window-period units here. You can see three in Massachusetts.

And -- the same format -- five window-period units in Connecticut and then the remaining 43 PCR-positives and 193 antibody-positives.

We also in this prospective study had one window-period unit in Minnesota.

The bottom line for what I have shown you so far is that four of every 1,000 donations in the four endemic states in which we tested are positive for markers of *B. microti*, either antibody, PCR, or both.

If I extend the testing data through the middle of March this year, we have tested over 95,000 donations, seen nine window-period collections, 69 that are antibody- and PCR-positive, 305 that are antibody-positive only, for a total of 383.

This is an epidemic curve of our positives, green being antibody-only, orange being antibody plus PCR, and red being the window period.

So, yes, we have seen window-period donations in the months of June through September, but have seen antibody- and PCR-positive units in almost every month of the year -- for PCR, all except for April, which is just an anomaly of data collection. So PCR positivity and antibody positivity occur year-round.

We have talked about positive predictive value. In our retrospective study we saw two false positives out of Arizona and Oklahoma. But combining the retrospective study with the prospective study, those are the only two false positives we have seen in all testing we have done. If you look at those two false positives, they occur in the late resolving phase. The way I have structured the slide is, window-period units, 9 of 9, all have confirmed, acute cases, all have confirmed, 74 of 74, early resolving -- these are now high-titer antibody/PCR-negative -- all confirmed, and we have only had two false positives, for an overall positive predictive value of 99.50 percent.

We followed donors up through the end of March. We have 419 positives. We followed 63 percent, or 262. They provided their first follow-up sample at a median of 7 weeks, their last follow-up at a median of 53 weeks, with a wide range. Donors provide follow-up samples, of course, when it is convenient to come to the blood center and provide a follow-up sample.

At the time of last follow-up -- and I will go into this in more detail -- 7 percent of our donors remained PCR-positive and 81 percent of the donors retained antibody at above the diagnostic cutoff of 1 to 64.

This table is not an eye chart, but it does show you all of the data that we have collected on donor follow-up for serology. On my far left, which I guess is your far right, it starts with the titers by antibody. The first row is window period, so those would be antibody-negative, the nine I mentioned, and then looking at decreasing concentrations of antibody. Along the bottom line, here are the 262 we followed of the 419. The duration of follow-up was 371 days. Fifty lost antibody titer, or 19 percent, over a period of time of 410 days. So these donors lost antibody in 410 days.

If we look at duration of time by year, then, 104 donors participated at 1 year or greater. That is 81 percent who retained antibody. For those 104 who participated and came back to donate again, we saw decreasing antibody titers a year or clearance of antibody, 35 percent by year 2, 12 percent by year 3, and 5 percent by year 4.

Looking at antibody now in a very illustrative subset of donors, these are our window-period donors. I show you antibody seroconversion to let you know that these donors are real. They were infected. They seroconverted. We did antibody titers below 1 in 64 to show you that even below the screening cutoff, these donors were antibody-negative. The lines in this chart show you the time to seroconversion. There is a vertical line dropped at one year and at two years. The parasite load at index for these donors was 144 to 1,635 parasite copies of DNA per milliliter. At the end of two years, you can see that one donor still retained antibody and three donors cleared. But it takes a long time. Many donors will take up to four years to clear antibody.

Now let's look at PCR clearance. We had 73 positives over the duration of time that we are looking at for follow-up. That is through March. Sixty-nine were followed and cleared PCR positivity -- that's 95 percent -- over a median time of 385 days. That is just over a year. Sixty-nine again went to negativity. The median time to negativity -- I should say we followed the donors for a median time of over one year, and 110 days were required to clear DNA, or 3.7 months. Ninety-five percent of the donors cleared DNA at a median time of 3.7 months.

If we compare that to what has been published -- and this is in a paper by Peter Krause that was published a while ago -- he compared subjects who were treated -- this is the survival Kaplan-Meier curve -- patients who were treated, which is in the solid black line, against patients who were infected with *Babesia*. These were asymptomatic, but they were part of a serosurvey. He compared treated individuals to untreated asymptomatic individuals to look at their duration of DNA clearance. As has been pointed out already by Susan Leitman, not all individuals will be cured by treatment.

Anyway, the point of this slide is to show you those who were asymptomatic. The mean for the duration of DNA in these individuals was 82 days, so about three months, so comparable to the 3.7 months that I showed you here.

But if we take our 73 donors and also do a Kaplan-Meier graph, 5 were censored because they remained PCR-positive during this study, 50 percent decline in 135 days, 75 percent decline in 223 days, and 95 percent decline at one year. That should be 95 percent, not 93 percent.

Looking at parasite loads at index, they range from 5, minimum, to 3 million, the interquartile range ranging from 40 copies to 3,000 copies of parasite DNA per ml.

Let’s talk about infectivity. I'm going to talk to you about infectivity in a hamster model, which is the most sensitive model for *Babesia*, and then talk to you about human infectivity.

We inoculated red cells from positive units into hamster pairs or into triplicate hamsters. Of the total that we have inoculated, 93, a third of them have been infectious, over 50 percent if the red cell unit was PCR-positive. So 50 percent of PCR-positives are infectious in hamsters. Looking at antibody-positive/PCR-negatives, we did get two positive donors that were positive in hamsters that were DNA-negative. However, these were only antibody high-titer units. In the experiments, I wanted to see first if high-titer units were infectious before I killed off a litter of hamsters with low-titer units.

Looking at the results, the first two are the window-period donors who are infectious in hamsters, ranging in copies from 400 to 1,100. The two in yellow show you the two that were PCR-negative/antibody-positive. One had a titer of 1 in 512. One, which did have demonstrable DNA by a more sensitive PCR test of 40 copies per ml, was high-titer at greater than or equal to 1,024 by AFIA.

Let me talk to you about some limitations of this. We are depending on the regions to send us these red cell units, and regions have more important things than doing my research -- that is, getting blood distributed. So they send us the red cells when they can. So my question was, is this 54 percent an underestimate because of the duration of time it took for our regions to send us units?

We looked at storage age of the hamster positives versus the negatives, and, yes, the DNA positives that were hamster positives had a range of 7 to 34 days -- that is, the time before inoculation -- versus the hamster negatives, which were 11 to 55 days. Those were significantly different.

Let's look at the PCR-negative units that were antibody high-titer. Remember, we had two of those. The two were inoculated into hamsters at 9 and 21 days. That was significantly shorter than the time period of hamster negatives of 6 to 69 days.

So these data are limited, but they do show that at least 50 percent of PCR-positives do infect hamsters, and infectivity in this case did last 34 days. We know from look-back studies and other studies that infectivity remains up to the duration of the lifetime of a red cell unit. That is 42 days.

This graph shows you by antibody titer, on the x-axis, the number of antibody-positive donors we have that decrease in number as antibody titer increases and then a concomitant increase in PCR reactivity as antibody titer increases. What I showed you for infectivity were only those antibody-positives that are 512 or 1,024, those two units. I didn't yet do infectivity studies on the lower positive, but this slide can serve as a warning that if we do widespread antibody screening, we will be deferring and notifying a lot of donors who probably have no current infection. So we would expect in titers of 128 or 256 to have infectivity rates far less than 4 percent.

Now looking at human cases of *Babesia*, no longer hamster, these are what I call our post-Herwaldt data. Since Barbara published her data, these are data that we collected through the Red Cross hemovigilance program. Of 82 cases of suspect TTB reported to us, we confirmed 47. Those are shown by the zip code if residence of the positive donor that was linked to the investigation. Those are the red dots. Again you can see that they are primarily located in the New England area and in the Upper Midwest. Yes, we have had donors who have traveled and products that have traveled. The dashed line going from Connecticut to South Carolina represents a donation that was transfused to a neonate in South Carolina and resulted in TTB. Had we been screening the full state of Connecticut, of course, that would not have occurred.

In this four-and-a-half-year period of time, we have had two traveling donors. One donor who camped in Maine that Barbara mentioned donated in Georgia, and then their product was transfused and recognized as TTB in Illinois. We also had a more recent donor who summers in Rhode Island and then went home to California to donate.

If we focus now on the donations that we have had since we have been doing prospective screening, of the 47, there were 29. Forty-one were reported to us and 29 were confirmed. A very important point about these TTB cases that were reported to us: None were screened at donation. So TTB resulted from unscreened units.

What I will show you on the next four slides is why they were unscreened. They either came from a region in which we don't do screening, a day of collection prior to the initiation of the IND, a county that is not included in the IND, a day that isn't included in the collections for which we test. But the most important columns here are the last three columns, showing you how we confirmed donor infection, either by antibody AFIA or DNA by PCR.

Here you can see the length of time that occurred between the index donation, the donation that caused infection, and when we followed the donor. Some of these are quite long. You can see that PCR positivity persisted and antibody persisted.

Here we had a PCR-positive donor who was antibody-negative who transmitted and was still PCR-positive 89 days later. Here we had an antibody-positive/PCR-negative. So these come in all flavors.

Just showing you more of these, the traveling donor -- California is the top -- Connecticut, New Jersey, Connecticut, New Hampshire, New Hampshire, New Hampshire. We have had a cluster of transfusion transmissions in New Hampshire. You can see follow-up results, when they were followed and what the results were.

Continuing on, Massachusetts, Connecticut, Maine, New Jersey, Massachusetts. You can see about the same pattern in all of these donors who were implicated.

The Red Cross 47 TTB cases since the 162 reported by Herwaldt, 29 since prospective investigational screening. The states involved, based on the donor residential zip code, included eight from Connecticut, with one product associated, seven in Massachusetts, five in New Jersey, three in New Hampshire, three in Maine, one in Minnesota, one in New York, one in California. That was the Rhode Island traveling donor. Seven of these 29, or 24 percent, occurred outside of the seven endemic states, one who was the traveling donor and three in New Hampshire and three in Maine.

Let’s do some risk calculations. Based on our collections over the 28 months since we have been doing prospective screening, outside of the seven endemic states, we have seen 7 of 10 million-plus donations, or 1 in 1.5 million donations, that transmitted TTB. I would also like to remind you that 1 in 1.5 million is the acceptable residual risk for HIV, HBV, and HCV. Travel, including New Hampshire and Maine, resulted in a risk of 1 in 1.45 million. We saw one travel case in those 10 million donations, so that risk is 1 in 10 million. If we look at the six that we saw in New Hampshire and Maine, that estimates to a risk of 1 in 50,000. When we looked at seven endemic states, of the 22 in 2.5 million collections over the 28 months, that was a residual risk of about 1 in 100,000. So 1 in 50,000 observed in New Hampshire and Maine was enough to trigger us to say that something has to be done in New Hampshire and Maine. So adding now those two states to the seven, the residual risk in the nine endemic states is 1 in 100,000, and outside of the nine endemic states, due to travel, it’s 1 in 10 million-plus.

What about recipient follow-up? I mentioned to you that we do look-back from prior units from positive donors. We have had 41 recipients who provided follow-up samples. Three have been antibody-positive, in a range from 64 to 256 titer. We did have one TTB case from a donor who was AFIA-positive, highly antibody-positive, and PCR-positive at index. The recipient did seroconvert and became antibody- and PCR-positive, retained PCR positivity through the course of study. The recipient was a 27-year-old female sickle cell patient who was transfused ten weeks prior to the positive index donation.

What risk did we put into the Association Bulletin for TTB? We got data from Rhode Island, which also screens using the IMUGEN IND. They had zero transmissions from 13,000-plus tested units, 11 transmissions from 352,000 untested units, for a residual risk of 1 in 32,000.

The data that we included in the Association Bulletin was zero transmissions of 60,000 and 10 transmissions of 200,000, or a residual risk of 1 in 20,000 untested units. This was not significantly different, that is, the difference in risk between tested and untested units, but did have an odds ratio of 6.3 higher chance of getting babesiosis from an untested unit.

But when we extended our numbers through the end of September, we had zero transmissions from 75,000-plus tested units, 14 transmissions from 253,000-plus untested units, for a residual risk of 1 in 18,000. That was statistically significant, showing that tested units carry a much lower rate of risk than untested units. The odds ratio of that is 8.6 for the risk in untested units.

That is shown in the table in this slide, 0 versus 14, a residual risk of 1 in 18,000.

Let’s put this in perspective for other residual risks from other things that we have taken mitigation steps for. I already mentioned to you that HIV, HBV, and HCV have a residual risk that is mitigated -- it’s in italics in this slide -- of about 1 in 1 million.

What about TRALI? We use our baseline of considering a success of about 1 in 500,000. Prior to mitigation strategies, we had a risk in plasma of 1 in 37,000 and in apheresis platelets, 1 in about 150,000. We have been doing interventions to try to mitigate those risks.

What about bacterial contamination of platelets? Unmitigated is 1 in 36,000. Even though the mitigation strategies we have struggled to implement, we still have a residual risk of about 1 in 100,000.

So where does that put *Babesia*? I showed you in the ten-county analysis of tested versus untested that we have a residual risk of 1 in 18,000. It’s probably time t do something about that risk.

We have also just recently published -- it will appear online very soon -- a cost-effectiveness analysis. It’s not the cost that I want to talk about, but it’s the number of cases averted by doing testing. We looked at a number of models in the four endemic states where we have been doing testing. We looked at universal PCR in those four states, universal antibody, and a combination of antibody and PCR. But as far as cost-effectiveness, what we found was that universal PCR actually was the most cost-effective. That’s because it has the highest specificity. It averted 24 to 31 cases per 100,000 units transfused. if we move to universal antibody plus PCR, the 24 and 31 increase to 33 to 42. What we concluded was that universal PCR in endemic states is an effective blood donation screening strategy at a threshold of $50,000/QALY.

Using a higher cost-effectiveness ratio, which went up to about 83,000, universal antibody and PCR is the most effective strategy.

Just looking at a table where we extended the four states to seven states, you don't need to see anything on this table except the bottom line. Per year, or 2 million red cell transfusions that we project occur in these seven states, universal antibody and PCR would interdict 131 units and prevent 11 deaths. Here you can see the other strategies.

Taking these same two columns and adding the number of false positives we have, this is the number of false positives, using a 99.98 specificity. This specificity was calculated using our retrospective study. Through prospective screening, specificity is undoubtedly higher than 99.98. But in any event, we would predict the loss of 398 donors.

Looking at the 131 cases relative to what the FDA predicts, their projections are significantly higher, whether you use a five-state, a seven-state, or a nine-state model. The delta there of about 200 units to 975 includes all the other states, with over 4,000 false positives, which I think is unacceptable.

In summary, prospective blood donor screening for *B. microti* is feasible and has resulted in 383 units being removed from the blood supply through the middle of March of this year. Sixty-nine of those were both PCR- and antibody-positive, including nine window-period units, for a rate of 1 in 10,000 -- again, 20 to 100 times higher than acceptable risks than our yield for HIV, HBV, and HCV. Screening has prevented transfusion-transmitted babesiosis. We know that 54 percent of our PCR-positive donors infected hamsters. Twenty-nine probable TTB cases occurred when and where no screening occurs. When we did our comparisons between screened and unscreened blood, they were significantly different, favoring the use of screened blood.

Our IND goal of qualifying a testing approach to reduce TTB in the blood supply appears to be successful. Our current testing model has converted from a research model that was supported by the Red Cross to a cost-recovery model, based on hospital orders.

With that, that ends my presentation, but I just would like to comment on the use of the FDA proposed model based on reporting of babesiosis cases through CMS. These comments are similar to those that you will hear from AABB and the ABC.

I believe it is inappropriate to use unvalidated data from CMS to model national screening policy for *Babesia* when the CMS data have not been fully investigated regarding their accuracy. The diagnostic tests used in the administrative database are variable, with differing and unknown rates of sensitivity and specificity. It is also unknown if the codes used contain diagnoses related to other tick-borne agents, which has been addressed by Richard Forshee -- that they took out California of *B. duncani*. But we still don't know if the database contains miscoding that would include *Borrelia*, the agent of Lyme disease, *Anaplasma*, and *Ehrlichia*.

It is also unreasonable to project 100 percent infectivity to donor units based on the FDA’s model when it is known and published that PCR-positive units are at least tenfold more infectious than PCR-negative units. The lack of specificity of the unvalidated assumptions used in the FDA model is the likely explanation for the higher number of projected TTB cases than actually observed from either reported cases of TTB or babesiosis cases reported to the CDC. If the FDA model is, in fact, valid, then one would expect to see many more reported cases of TTB, including in states that have never reported a TTB case. Most states listed in the CMS model would not even expect to see one case of TTB in a given year.

The FDA acknowledges many of these limitations in the appendix and has asked -- presented today, but still uses these unpublished and unvalidated data as a basis from which to recommend policy.

Lastly, it should be noted that not all investigational tests have specificity of 99.98 percent.

Thank you.

DR. JACKSON: Thank you very much, Sue.

Our next speaker is Andrew Levin. Dr. Levin is with Immunetics and will be speaking on “Screening with an Investigational Enzyme Immunoassay for *Babesia microti* Evaluated in an IND Study on US Blood Donor Populations.”

Agenda Item: Screening with an Investigational Enzyme Immunoassay for *Babesia microti* Evaluated in an IND Study on US Blood Donor Populations

DR. LEVIN: First, thank you very much to the FDA and the Blood Products Advisory Committee for inviting me to speak today.

I’m going to be reporting on the results of a study that we carried out evaluating an investigational enzyme immunoassay for *B. microti* screening in blood donors. This study was carried out with a number of partners: Creative Testing Solutions, which carried out most of the testing, Blood Systems Research Institute, United Blood Services. Donors were provided through New York Blood Center, UBS, Memorial Blood Center in Minneapolis, and clinical cases through Yale and Tufts.

I don't think I need to go through the first couple bullets here. Going to the third bullet, Immunetics has developed an investigational EIA which is intended for blood screening for antibodies to *B. microti*. I think it is well known that almost all infected individuals develop an immune response to *B. microti*. As we have just been hearing, there is a window period and a convalescent trail for antibodies.

We carried out with our partners a pivotal study under an IND from the FDA to evaluate the use of this investigational EIA in screening US blood donors. The analyses in this presentation are not necessarily final because the study is not quite wrapped up. However, I think they represent the vast majority of data we have to date.

This begins about 15 years ago or so when we began working with peptide antigens derived from *Babesia microti* genes that had originally been described by David Persing and colleagues at Mayo Clinic and subsequently Corixa Corporation. That group identified an immunodominant gene family called BMN1 which was described as including multiple individual genes and protein antigens. We began to work with that family of proteins and subsequently refined the sequences that were used to generate peptide antigens over a number of iterations involving an epitope scanning approach. In the end, the specific peptides that we worked with in our assay were not identical to those described by the Persing papers, but from the same gene family.

We developed a microplate ELISA. This is a 96-well microplate ELISA format in which four peptides are immobilized to the bottom of the microplate. Serum antibodies are detected. Binding of serum antibodies to the peptides is detected by an adding an anti-human IgG and IgM enzyme conjugate and an enzyme substrate. This is formatted as a kit. The turnaround time is about 3 hours. Being a 96-well ELISA plate, it is suitable for use for either manual operation or high-throughput automated operation on all of the various platforms that perform ELISA testing on microplates.

In our earlier studies prior to the IND that I am reporting here, we looked at the sensitivity and specificity of this ELISA. The specificity was evaluated in a population of teenage blood donors from Arizona, so these are individuals who are very unlikely to have traveled outside the state and represent a pretty clean population. The graph here shows ELISA absorbance versus blood donors. We see that they are pretty clean in this ELISA, enabling us to draw a provisional cutoff line here. By comparison, looking at clinical cases from symptomatic *Babesia* patients from mainly the Northeast, the majority of those patients were clearly at much higher absorbance levels. The average absorbance level for symptomatic patients is quite a bit higher than for asymptomatic patients. But there were two PCR-positive samples which were below that cutoff level.

This was prior to the IND study. Then we organized this study. The donor collections were carried out between August and November of 2013. This involved donors from three regions: New York, Minnesota, and New Mexico, New York and Minnesota being endemic regions from which we collected somewhat over 13,000 and over 4,500 donors, respectively, and then New Mexico as a non-endemic region, from which we collected about 8,500 donors.

Samples from all these donors were processed at Creative Testing Solutions laboratories in several states. Those laboratories carried out testing with the investigation EIA. They followed a conventional reactive algorithm. Samples were tested initially. Initial reactive were retested in duplicate and repeat reactive were two out of three. Repeat reactive were then subsequently tested by format that is in parallel. RBCs from the same donor were tested by PCR at Blood Systems Research Institute.

This actually was two PCR assays, with two separate sets of primers to add additional specificity. The first PCR assay was developed by Evan Bloch et al., published in *Transfusion* in 2014, I think. That assay -- the limit of detection is 13 gene copies per 2-ml blood sample at the 95 percent level or two copies at the 50 percent level. Samples which were positive by that PCR were rescreened on a separate PCR, which was similar to the assay developed by New York State laboratory, published by Thiel (phonetic) et al.

In parallel with that assay, samples were evaluated by blood smear, by conventional IFA, and by immunoblot. These assays are all RUO and validated internally.

This was a linked donor study in which donors were consented and they were informed of results and deferred if positive on the ELISA.

In parallel with that, we conducted a smaller unlinked donor study, actually over two seasons, in which we collected a little over 2,500 donors from a highly endemic region, Nassau County, New York and Suffolk County. These samples were screened in parallel by the ELISA and conventional IFA, PCR, and blood smear. The aim was to investigate the frequency of samples which were positive by any of these techniques, but independent of EIA status.

Looking at the results over time, this study was carried out from the end of July until mid-November. The green peaks show sample collection volume flowing through the testing laboratories. It’s sort of up and down, with a minimal point here at Labor Day weekend. It looks like people don't like to donate blood around Labor Day weekend.

They have better things to do. But other than that, the green peaks were fairly consistent overall. The EIA results showing repeat reactive rates are in red and PCR-positive shown in blue here. What you can see is that over this three- or four-month period, the rate of EIA reactivity sort of bumped up and down a little bit. It did not follow any clear pattern. Certainly it didn’t follow the pattern of clinical infection, which would have peaked in midsummer and decreased towards autumn. We found a spike even at the very end of the period, around November 15. Maybe that represents hunting season. We don't know.

PCR cases likewise, in blue, were somewhat bumpy, and there is no clear pattern. We did again find a spike in PCR cases towards the very end of the period.

Looking at the distribution of absorbance values amongst these populations, in the negative population, most of the samples are below the provisional cutoff. We did look at the data after the study and are in the process of revising the cutoff to optimally separate positives from negatives. The dotted line here shows our proposed revised cutoff. At this level, we have the majority of the non-endemic cases in New Mexico below the cutoff, but some floating above. In the endemic regions, New York and Minnesota, there is clearly a cluster of cases which are well above the cutoff. In the clinical group -- these are symptomatic *Babesia* patients -- the vast majority are above the cutoff, except these two cases here, of which one turned out to be antibody-negative by other methods and the other was about halfway to the cutoff and rather low in reactivity by other methods.

We found what we believe to be nine credible PCR-positives in the endemic region, none in the non-endemic region, New Mexico, but eight PCR-positives in New York and one in Minnesota. Of these nine PCR-positives, six of them met the criteria of positivity by both the screening PCR and the confirmatory PCR. Three others were positive by at least one of the PCRs and equivocal by the other. Based upon the other evidence, we consider them probable actively infected cases.

All of these cases showed high IFA titers, except for this one at 512. The others were at 1024. They were all positive by the Western blot.

Conversely, looking at the clinical cases, if we looked at the 52 clinical cases, 50-odd of those were positive by EIA. That shows that the EIA detects the vast majority of PCR-positive cases.

We used Western blot for additional characterization of EIA-reactive. The basic findings by Western blot were, as expected, blood donors that are positive by EIA and PCR resemble clinical cases. The band patterns are fairly similar. Here is a New York blood donor. It looks fairly similar to the clinical cases.

However, an interesting finding was that in New Mexico, the non-endemic region, where we had a small number of EIA-reactive, no PCR-positives, but of those EIA-reactive, a small number of those had Western blot patterns which look very, very similar to a true clinical case or a reactive blood donor from the endemic region. That suggests that there are cases in non-endemic regions, such as New Mexico, which may be true babesiosis, *B. microti* infections. Whether or not these individuals acquired them outside the state we are tracking down now. But certainly it is possible that they acquired them elsewhere, or it’s also known that there are other species of *Babesia* that inhabit these areas which may not be pathogenic in man. However, they are highly prevalent in the animal population. We don't know what their Western blot profiles might be, but that is another interesting possibility.

Looking at the numbers from the IND study overall, from the endemic regions, the EIA produced 45 repeat reactive, for a rate of .25 percent. This is summarizing both New York and Minnesota donors. The New York rate actually was about double that of Minnesota. New York itself was about .29 percent and Minnesota about .15 percent in repeat reactive donors. The non-endemic New

Mexico region produced 11 repeat reactive, for a rate of .13 percent. Following the further assays, of the 45 repeat reactive from the endemic region, as I said, nine were positive by PCR methods, at .05 percent total, we had no blood smear-positives, IFA produced 26, and Western blot 27 reactive. In the non-endemic group, again no PCR-positives, no blood smear-positives, and four samples positive by either IFA or Western blot.

In the unlinked portion of the study, of these 2,500 samples from endemic regions in New York that were tested by all four methods in parallel, we found 29 repeat reactive by ELISA, or 1.15 percent. Of those 29, one was positive by PCR -- actually, I shouldn’t say of those 29. There was one sample positive by PCR, which was also positive by ELISA at a signal cutoff of over 4 and a relatively high IFA titer. There were samples found positive by IFA, but none positive by blood smear. This suggests that, at least on this scale, that level of sample was found positive by both EIA and PCR. We do not have the ability to process a large enough volume of samples by PCR to be able to run PCR on all of the 26,000 donors, however, so we couldn’t extend that study/

Overall, our preliminary IND study results show 45 reactive donors in the endemic group, of which 18 were what we would call likely false positives because they were negative by Western blot criteria, and 27 likely true positives, which were positive by Western blot criteria. That leads to a calculated positive predictive value of 0.6, 60 percent, in the endemic region. Obviously, if we had confirmed these samples with a second method, such as Western blot, and put that into the calculation, that number would go up. In the non-endemic group from New Mexico, we found 11 repeat reactive, of which seven appeared to be false positives, four appeared to be true positives, for a positive predictive value of .36.

Specificity: Out of the non-endemic donor group, where we removed the Western blot-positives and calculated based upon the Western blot-negatives, specificity for the entire group was 99.92 percent. Likewise, sensitivity, calculated based upon gold-standard clinical cases which were positive by PCR or blood smear, independent of serology -- of 52, we detected 50, for a sensitivity of 96.15 percent. That number, of course, might vary depending upon additional cases which may be either window-period or, conversely, convalescent trail.

This study can be summarized by saying that:

• ELISA is effective in detecting the vast majority of PCR-positive samples, based upon performance on the clinical babesiosis samples.

• ELISA yields a false-positive rate less than

0.1 percent overall among blood donors in the whole study.

• The positive predictive value of the EIA is about 60 percent in endemic areas.

• The yield of PCR-positives per EIA-positive is about 20 percent in endemic areas.

• Based on that, EIA is likely to be a cost-effective screening tool for *B. microti*, which has been reported in various publications, such as the one quoted here, which came from *Transfusion* last year.

Further steps towards evaluation of this EIA in screening blood donors: We are working with Blood Systems Research Institute, which is managing a study which enrolled EIA-reactive donors tested under the IND for a follow-up period of one year to measure kinetics of the IgG/IgM EIA titer to *B. microti*, in parallel with PCR testing to monitor infectivity. When those data are complete, we expect to be able to add them to the body of data informing further discussions of deferral and reentry criteria.

As the IND that we are operating under s continuing, we expect that screening with this EIA will be available through our partner, Creative Testing Solutions, to blood centers that are interested.

Being an ELISA in microplate format, of course, production is scalable. We are prepared to ramp up production to suit whatever volume needs come to be. More than half a million tests per week can be produced. We are aiming to suit whatever the need might be.

Of course, automated platforms can run the EIA for high-throughput screening. We have software solutions to facilitate that.

Lastly, I would like to thank the group of people who made this study possible. Creative Testing Solutions carried out all the testing. Phillip Williamson is here today. Mike Busch and Evan Bloch managed the study out of BSRI, Beth Shaz and Debbie Kessler out of New York Blood Center, Jed Gorlin from Memorial Blood Centers, and our clinical collaborators, Peter Krause and Sam Telford. Lastly, I would like to acknowledge support from the NHLBI, which funded the study.

Thank you very much.

DR. JACKSON: Thank you very much, Dr. Levin.

For our final speaker on this topic, Dr. Sanjai Kumar will come back to present considerations for testing blood donations.

Agenda Item: Considerations for Testing Blood Donations for *B. microti*

DR. KUMAR: Good morning again.

The intention here is to sum up FDA considerations. Each consideration that you will see here is tied up in a way so it will guide you to look at the FDA questions that we will be presenting later on and help you reach those decisions.

Based on these current considerations, FDA is seeking advice on strategies for implementation of testing of blood donations of evidence of *Babesia microti* infection when licensed tests become available. Data that we have seen so far, again based on our CMS data, indicate that about 93 percent of current babesiosis cases are located in 15 states plus Washington, DC. However, babesiosis has been reported in all states plus Washington, DC, except the state of Wyoming. So FDA is asking the committee whether antibody testing should be nationally based and year-round, and if NAT testing should be limited to certain states only.

To elaborate upon that, based on the FDA analysis, we are presenting these three options for the committee to consider:

• Whether NAT should be limited to the five highest endemic states — Connecticut, Massachusetts, Rhode Island, New York, and New Jersey.

• Nine states that are all known endemic states, these five states plus the states of Minnesota and Wisconsin, New Hampshire and Maine.

• The third option that we want you to consider is 15 states plus Washington, DC. We have chosen this option to be considered because it presents the largest risk capture with the smallest number of states that need to be considered — Connecticut, Massachusetts, Rhode Island, New York, New Jersey, Minnesota, Wisconsin, New Hampshire, Maine, Maryland, Washington, DC, Virginia, Vermont, Pennsylvania, Delaware, and the state of Florida.

That is the consideration in term of NAT testing.

You saw this in Dr. Forshee’s presentation. It simply just gives you a visualization of the states where we are asking you to consider NAT testing, in the Northeastern states here plus in New Jersey. The nine states here include the seven states here plus the state of Wisconsin and Minnesota. The 15-state strategy here also includes Florida.

In addition to antibody and NAT testing, FDA is also seeking advice on a temporary deferral period for donors who have positive test results for *Babesia microti*. This is based on limited data coming from the epidemiological studies for considering an appropriate deferral period based on antibody duration as are available in the published literature. Results from IND studies conducted to determine the performance of *Babesia microti* donor screening tests may contain information useful for establishing an appropriate deferral period. I think you saw a lot of data from Dr. Stramer’s presentation looking at the categorization of the duration of antibody responses in donors where follow-up testing was performed after index testing.

Our considerations for antibody testing -- and some of this I touched upon in my previous presentation, but I think I would like to go over this one more time again. Antibody testing:

• Detects the vast majority of parasitemic donations. But it seems like we saw evidence of one window case here which remained PCR-positive, but did not seroconvert. So those scenarios may also exist. But antibody should detect, if sensitive enough, low-grade, chronic infections that may be missed by NAT.

• Will fail to detect infectivity prior to seroconversion. I refer back to the model I showed you of infection in my previous presentation.

• Presence of antibody does not indicate active infection.

• Seroreversion, expected in the absence of chronic infection or new infection -- and we did see some data where seroreversion does occur -- may allow donor reentry based on a donor’s negative antibody test results after a deferral period.

Our considerations for NAT testing which we would like the committee to keep in mind while making decisions:

- NAT, if sensitive enough, can detect window-period infections prior to seroconversion. I think that is a very important consideration in endemic areas.

- May fail to detect low-grade, chronic infections below the threshold of detection sensitivity and early window-period cases. Those will be certainly missed before the parasitemia has not risen high enough

- Limited ability to differentiate between active and cleared infections.

A bit more consideration on antibody as a marker for *Babesia microti* infection. It doesn’t matter which data set you look at, it seems like about 20 percent of seropositive individuals, both for epidemiological studies and clinical studies now, were found to be parasitemic by PCR. So these seropositive, about 20 percent were parasitemic. *Babesia microti*-specific antibodies may clear within one year -- at least a subset of donors -- although in some cases antibodies may persist for several years. That is where we need more data to make an informed decision about what subsets of seropositive donors will become seronegative within what period of time.

Clearance of parasitemia is often associated with decline or resolution of antibody titer. The persistent *B. microti* antibody levels may be attributed to prolonged asymptomatic infections or reinfections. We don't know. Maybe some have prolonged memory responses as well.

Looking at the available data, I have tried to put only the data here where only information was available, which is very limited. Looking at this early history by Ruebush et al. in 1981, they used IFA to measure antibody responses. Where we could find the clean data, four out of six *B. microti*-infected individuals had a significant loss of IFA titer at 13 months after the onset of illness. So there is a significant loss. It does not mean it comes down to baseline, but it comes to titer 64 or lower. Then we have to consider where the cutoff is set to consider somebody seroreverted or not.

The next history is the Peter Krause history, which everyone has referred to, a *New England Journal of Medicine* study. In 12 patients, *Babesia microti* DNA lasted for three months or slightly longer, while the antibody levels either returned to baseline or significantly declined at 13 months after the diagnosis.

So there is a sort of parallel between the presence of parasitemia with a few months delay and then decline of antibody titer. That is not to say that this is always the norm.

A more recent study by David Leiby. For the six donors in this study where the follow-up data was available, these six donors became seronegative within six to nine months of being parasitemic. They became seronegative within six to nine months after their parasitemia was gone. But three donors remained seropositive over a three-year period despite receiving anti-*Babesia* treatment. I don't know what to make of it, really. Either the antibodies persist longer or the treatment was not completely successful, which is also a possibility.

In this Moritz study, 2015, which I think is in press now, 85 percent of donors retained antibodies at titers of 64 or greater at the last follow-up. Median was 262 days, interquartile range was 84 to 435, and overall range was 17 to 1,273 days. But you have heard a lot more detail here from Sue Stramer’s presentation. So maybe that is a slide that should be put up here when we are considering this.

I’m going to summarize some of our risk analysis, which Dr. Forshee so elegantly presented. He showed you the results from the scenarios, 12 different combinations of testing scenarios. But I am going to summarize the results. Only the baseline testing in every case was done by antibody in all 50 states. Then we are added NAT testing on top of that in three different testing scenarios.

If antibody testing is done in all 50 states, you will see about a 91 percent risk reduction for TTB. If you add NAT testing in the five highest endemic states, we will gain close to 3.8 percent, 3.6 percent risk reduction. If you perform NAT testing in nine states, you gain some increased reduction. If you include 15 states, it bumps up to 95.7 percent. But if you perform NAT testing along with antibody testing in all 50 states, the difference is not that great between 15 states and 50 states.

Looking at the same data in terms of number of positive units that would be interdicted: By antibody testing alone in 50 states, we have around 940 units would be interdicted. If you add NAT testing -- we presume these are all window cases and probably not loss of seroconversion here -- you increase from 939 to 975 cases. If you include NAT testing, you pick up an additional four window cases here. If we increase it to 15 states, those numbers go up to 983. But again, similar to risk reduction, the gain is not that significant if we do NAT testing in all 50 states.

Looking at the positive predictive value, the PPV is 18.6 percent if antibody testing is done in all the states, but PPV does increase, because we are picking up these window cases, to 19.1 percent with five states. The PPV does not improve if we conduct NAT testing in nine states. A slight gain if we do NAT testing in 15 states, but there is no change whether we conduct NAT testing in 15 states or all 50 states here.

I would like to point out here that we do acknowledge that a positive test result does not necessarily indicate infectivity.

I would like to leave you the questions again. I will repeat the questions one more time: Do the available scientific data and FDA analysis support the concept of nationwide, year-round testing of blood donations for *Babesia* risk by antibody-based test? If not, please comment on alternative options that FDA should consider, including limitation of antibody testing to specific states. The slide from Dr. Forshee’s presentation does consider different permutations and combinations of testing scenarios.

The second question, again coming back to implementation of NAT testing: In conjunction with antibody testing in all 50 states, we are again offering these three different options to be considered: in five state, NAT testing along with antibody testing, in nine states, these states being listed here, and in 15 states. Please comment on alternative options, if these scenarios are not acceptable, that FDA should consider for blood donation testing by NAT.

Coming back to the third question: Based on the limited available data plus the results you see from Dr. Stramer's presentation, please comment whether it would be appropriate to apply a time-based deferral for those donors who have *B. microti*-positive test results. If so, please advise on a suitable deferral period for donors who had *B. microti*-positive test results.

With that, I would like to acknowledge the people who were involved with this work over a long period of time and helped prepare this presentation.

I will stop there and take questions, if any.

DR. JACKSON: Thank you very much, Dr. Kumar.

We do have a few minutes for questions for the speakers. We will have an hour for open committee discussion as well. Are there questions for any of the speakers, not just Dr. Kumar, at this point?

Agenda Item: Questions for Speakers

DR. LEITMAN: I have a question. The presence of antibody in true infection with subsequent seroreversion, that is not a neutralizing antibody in terms of subsequent infection, so a person can get infected twice?

DR. KUMAR: In endemic areas we have to always consider reinfections, which may happen. But the important point here is what the antibody response is, the antibody titer at the time testing is done, because we can never avoid the issue of reinfection. I guess what we can consider if seroreversion has occurred at the protracted(?) chronic infection, leading to loss of antibody titer.

DR. JACKSON: I have a question for Dr. Stramer. In your presentation, it sounded like in the end you were suggesting seven state endemic areas for testing?

DR. STRAMER: No, nine. This is seven plus two, based on a threshold of TTB of 1 in 100,000.

DR. JACKSON: And those nine were the same as were just presented?

DR. STRAMER: Yes, they are the seven traditional plus New Hampshire and Maine.

DR. KUMAR: So we are talking about the same nine states.

DR. NELSON: What about Pennsylvania? Was that included? It seems like Dr. Herwaldt suggested that there may be emerging -- or there may be data that hasn't been reported from Pennsylvania that may be a risk area.

DR. STRAMER: The most important thing that I can comment about on a regional model is when we expand. So we are at nine, but as many speakers have said, this is a very dynamic scenario. So we have to be vigilant. Certainly Pennsylvania is on the hit list. We have had since Herwaldt three TTB cases in Pennsylvania, although they were prior to the period of time that I reported it. But looking at all of Barbara's together in the 35 years or so that she presented, there were 11 TTB cases from travel. You are talking about Pennsylvania being endemic, but just let me finish. For the travel, it turns out to be .3, or a third of a case, per year. That is pretty much like the residual risk that I showed you attributable to travel of about 1 in 10 million.

Pennsylvania is definitely on the line.

DR. KUMAR: I would just like to respond to that. Although Pennsylvania is not in our nine states, we have under question inclusion of Pennsylvania. So it’s not off the table.

DR. JACKSON: Other questions? Dr. Ragni.

DR. RAGNI: In the cases where the EIA is positive and the PCR is negative and you are thinking it might be a low infectivity, was Western blot looked at in those particular cases? In other words, is there any way to prove that or to confirm that they are truly infectious, even though you are thinking they are?

DR. KUMAR: I am not sure if I am the best person to address that. Dr. Stramer or Dr. Levin may be able to comment on that better.

DR. STRAMER: That’s why I showed you animal infectivity studies, so we can understand the difference. Of PCR-positive donors, 54 percent of those were infectious in a hamster model. Of the units we looked at that were

PCR-negative, just with high-titer antibody -- not all antibody-positives -- only 4 percent, or two, were positive. So there is much lower infectivity in antibody-positive subjects.

DR. LINNEN: It’s all going to depend on the sensitivity of the test and how many replicates you are capable of performing. If there is enough volume -- in my presentation for the public session, I will talk about how the sensitivity of the test really changes the ability to confirm low-level positive samples.

DR. BASAVARAJU: Let’s say we vote on five, nine, or 15 states. What would be the FDA’s process for, in the future, deciding to add or subtract states based on changing epidemiology?

DR. KUMAR: The normal process is that we will consider the committee’s recommendation, where we have asked as a direct question or a comment. Then we will have our own deliberations. Then we write a guidance document. That guidance document becomes effective once the licensed tests become available.

DR. BASAVARAJU: But let’s say two years from now or something, a new state that wasn’t added on initially becomes identified as hyper-endemic. Could FDA just make the decision on its own to add that state?

DR. KUMAR: We have to rely on the tools that are available to us. Currently some testing under the IND has happened. But then we have to wait upon the availability of licensed tests.

DR. DURKALSKI: Can someone comment on the feasibility differences between the five, nine, and 15 states -- the feasibility of actually implementing this?

DR. KUMAR: In terms of ability to test?

DR. DURKALSKI: In terms of the states being feasible to do this, maybe not so much on a cost level, but just the differences between choosing nine versus 15, I guess.

DR. KUMAR: From the FDA’s perspective, the feasibility -- we look at the other models, like for hepatitis viruses, HIV. Every single blood unit is screened throughout the country. It depends on the benefit-risk analysis, and where the risk lies. That’s why in 2010 the concept of regional testing was already endorsed by the committee. It depends on what the need is, what the recommendations are. But universal testing is done for other pathogens. It’s not uncommon.

DR. LEITMAN: I have a question for Dr. Stramer. On the table that Dr. Forshee distributed, which is very informative and clear, the last set of data on donors with false-positive results -- the FDA extrapolation for the entire country if serologic testing was implemented was only 2,400 per year, which is very small. The numbers you suggested from your sentinel areas seem much higher.

DR. STRAMER: In the issue summary, the 4,160 was what was in the 50-state model of false positives. They assumed a 99.98 percent specificity.

As I mentioned, for all of the testing we have done, for 100,000-plus donations, we have only seen two false positives. That was in the retrospective study, both at very low cutoffs. One wouldn't even be positive in our screening. So the specificity we see is very, very good and doesn't translate to those numbers.

DR. FORSHEE: If I could briefly comment on that, I think the difference between the 4,160 and the 2,000 number that you see is that here I am showing donors, and in the issue summary we were also showing false-positive units, which gets multiplied by 1.7 because of multiple donations per year. That's one of the reasons for the differences between those two figures. Again, we were assuming a 99.98 percent specificity for the model here. If in fact we are seeing better than that in the real world, then those numbers will be less.

DR. NAKHASI: My name is Hira Nakasi, from FDA.

I just want to respond to Dr. Basavaraju's question: What would be the process if tomorrow morning you find cases in other states -- how to change the policy.

Basically we will look at the data, as the deliberations happen here, and if there are cases reported outside those states, then obviously we will have to recommend testing in those areas, through guidance or other processes. But basically the issue is to look at the data before we make the decision.

DR. JACKSON: We have time for one more question. Dr. Simon.

DR. SIMON: I would like to ask Dr. Stramer -- sorry if I missed it -- based on your data, do you have a recommendation related to the last question for a deferral period?

DR. STRAMER: Let me just first respond to the question of expanding states again. This time, as Paul Hollande used to say, I’ll put on my AABB hat. As chair of TTD, we will monitor cases of TTB in states in the United States and come out with recommendations based on what CDC reports and what we see for TTB cases. I don't think we consider the CMS model in what we would want to do for expansion of cases.

Toby, your question again?

DR. SIMON: Based on your data --

DR. STRAMER: Oh, reentry. I said 81 percent of individuals --

DR. SIMON: Deferral period.

DR. STRAMER: -- still retain antibody at one year. So having a time-based deferral -- what we have bandied around by email is that you could have a one-year deferral and then, if you are antibody- and DNA-negative, you could be reentered, or maybe something like a two-year deferral and have no testing. To have a one-year deferral and have donors come back in and be antibody-positive is not constructive. So it would have to be greater than a one-year deferral.

But 81 percent of donors still retain antibody and about 7 percent still retain DNA.

Agenda Item: Open Public Hearing

DR. JACKSON: At this point we will proceed to the open public hearing, and I will read this statement.

Welcome to the open public hearing session. Please state your name and your affiliation, if relevant to this meeting. Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the open public hearing session of the advisory committee meetings, FDA believes that it is important to understand the context of an individual’s presentation. For this reason, FDA encourages you, the open public hearing speaker, as you begin to state if you have any financial interests relevant to this meeting, such as a financial relationship with any company or group that may be affected by the topic of this meeting. If you do not have any such interests, also FDA encourages you to state that for the record. If you choose not to address this issue of financial relationships at the beginning of your statement, it will not be preclude you from speaking and you may still give your comments.

With that in mind, I think our first speaker on the list is Allene Carr-Greer from the AABB.

MS. CARR-GREER: Good morning. Thank you, Dr. Jackson. Allene Carr-Greer. I'm presenting on behalf of AABB. I do not have any conflicts.

Thank you for the opportunity to provide these comments today.

As early as 1989, AABB initiated a recommendation for indefinite deferral of blood donors who provided a history of babesiosis, and we followed that in 1999 with a standard in the Standards for Blood Banks and Transfusion Services. This was the 14th edition, but that same standard remains in effect today, with the 29th edition, as we get ready to move into the 30th edition.

The current AABB donor history questionnaire contains the related question. It reads: Have you ever had babesiosis? But limited data indicate that this question is only marginally effective at preventing cases of TTB.

In 2008 the AABB Board of Directors established the TTD *Babesia* Work Group to provide leadership, with the goal of analyzing risks to the US blood supply for TTB and developing scenarios to mitigate the risk. In 2010 AABB presented a statement to this committee in support of the concept of regional testing of blood donors for *B. microti*. At that time we acknowledged the difficulty in determining which geographic areas have sufficient risk to warrant testing, but we did recommend that a regionalized testing approach should be defined by highly endemic areas identified through sound scientific studies of donor prevalence or locally acquired incident infections from donors or in recipients.

In the intervening years, several IND studies evaluating *B. microti* screening tests in the blood donor setting have concluded. Some are ongoing in various fashions. We understand that the study sponsors have or will soon submit their product to the FDA for licensure.

AABB has spent time trying to understand what would be current definitions for regional approaches. To that, we have identified that the regions to be included as high-risk presently would be the entire states of Connecticut, Massachusetts, Rhode Island, New York, New Jersey, New Hampshire, Maine, Wisconsin, and Minnesota. The selection of the seven states -- that is, Connecticut, Massachusetts, Rhode Island, New York, New Jersey, Wisconsin, and Minnesota -- is based upon a well-accepted consensus among public health experts for the relatively high incidence of human babesiosis cases by vector transmission -- that's 95 percent of the cases reported nationally to the CDC in 2013 -- as well as documentation of TTB in the extensive published CDC case series that Barbara told us about today.

TTB cases by state, based on the implicated donor's state of residence, were reviewed by the our TTD *Babesia* Work Group. After that review, AABB has included the two additional states, New Hampshire and Maine, due to the high incidence of TTB in those states in very recent years.

Again, vigilance is what has been talked about this morning. With the concept of regionalized testing in mind, it's our belief that the nine states should be subject to change as additional data become available. The AABB TTD *Babesia* Work Group will continue to survey for the occurrence and frequency of TTB in other states, particularly in states bordering those identified above, and will remain vigilant to increases in vector-borne illness in a new area in the absence of reported TTB cases.

Since 1989, AABB has encouraged the use of available methods to mitigate the risk of TTB, and in the past year, AABB has extensively reviewed the potential for TTB using a risk-based decision-making framework and utilizing data that has been carefully investigated for relevance to the policy being discussed today. With the availability of licensed screening tests -- when they are available -- for *B. microti* in blood donors, AABB recommends the following with regard to strategies for implementation of testing for *B. microti* in blood donors. That would be year-round regional testing of all donations of transfusable red cell products, specifically in the nine states described above, with the inclusion of additional states based on scientific data or exclusion of specific areas within a state as described in an area I will get to below.

We encourage FDA to move expeditiously to review submissions of test applications as they are submitted. As the agency develops current thinking around the use of *B. microti* testing, it should consider the use of testing systems that have high positive predictive values and low rates of false positivity to conserve blood availability, and low rates of false negativity leading to optimal safety.

AABB encourages the development and approval of a supplemental test and/or reentry algorithms to allow for donor reentry as expeditiously as possible, as it is important that the number of donors who are not infected with *B. microti* yet who are deferred due to false positive reactions remains low.

As always, there are other considerations. In consideration of the impact of the implementation of the testing described above on blood centers and hospitals, and specifically in the context of limited health-care resources, AABB believes that extensive advocacy efforts are required to ensure that *Babesia* testing can be accomplished without disruptions to the availability of blood and components in the affected states. AABB will develop, in coalition with other like-minded organizations, an advocacy plan with the goal of pursuing broad-based support that ultimately will allow for balanced approaches toward appropriate reimbursement policies, with an immediate goal to identify and secure cost-recovery funding for implementing regional testing recommendations. Likewise, AABB encourages the FDA, through its participation on the ACBTSA, and other Department of Health and Human Services committees to recognize the financial impact of additional testing and support appropriate reimbursement policies.

Because the incidence of babesiosis may differ within a state, the omission of testing within parts of a state should only be based upon the accumulation of donor screening data demonstrating the absence of significant risk in those areas. Such data should be reviewed with public health officials for consensus prior to eliminating any given state areas.

AABB acknowledges that regional testing will not completely mitigate risks for transfusion-transmitted babesiosis. Based on data and presentations we have heard at today's meeting, and as described in the FDA's issue summary, screening of blood donors for *B. microti* is a definite step forward in efforts to mitigate TTB. We encourages HHS to work with all agencies in the public health arena to mitigate babesiosis at its source.

AABB does not support use of this CMS database as an appropriate set of data upon which to base policy for screening blood donors for *B. microti* for the reasons noted in the issue summary appendix:

• The diagnostic codes do not necessarily represent incident codes.

• The unavailability of information to confirm potential TTB cases.

• The lack of clinical information to identify *Babesia* species.

I thank you.

DR. JACKSON: Thank you.

Next we have Dr. Jeffrey Linnen, from Hologic and

Grifols.

DR. LINNEN: My name is Jeff Linnen. I am from Hologic in San Diego. We are in a partnership with Grifols for the commercialization of NAT blood-screening assays.

I’m going to talk briefly about our very early-stage work in developing a NAT assay and then talk about something that might be a little bit unexpected, the possibility of testing in pools.

I am going to cover three things, very briefly:

• The design goals for a transcription-mediated amplification assay. This work is done for development of an assay on what is called the Procleix Panther System, which is not available commercially in the US, but is in wide commercial use in Europe and other places in the world.

• I will talk about analytical sensitivity.

• Some very preliminary data on clinical sensitivity and the detection of *Babesia* in red blood cell specimens, and then some information on the effect of pool size on sensitivity.

I would just like to outline the preliminary assay design and the goals for the feasibility phase of assay development. For this particular agent, we needed to develop a new sample preparation method that was compatible with our current NAT methodology, TMA. It also had to be compatible with our current automated instrumentation.

I am a little bit surprised with the work that has been done so far with *Babesia*. The approach that we are taking is to target all of the species of *Babesia*. That is the general approach. Unless you want to exclude a sequence, we would like to be able to detect all of the species that are clinically relevant.

The sensitivity is very similar to NAT tests that we have previously developed -- 10 to 30 copy per ml sensitivity. The specificity, which we have heard a lot about today, would have to be very high, close to 100 percent. Then we want to demonstrate the effectiveness in various pool sizes, four, eight, and 16 donations.

Just briefly about the way the samples are prepared: What we have done is we have created a simple step prior to putting the sample on the automated instrument. It’s pipetting of one reagent. It’s a proprietary licensed solution. It releases the RNA target that we are detecting and preserves that target. So it’s a single pipetting step. What is shown here is just the outline.

Since we are in the early stages of development, this process is scalable in terms of the volume of the sample. Most of the work that we have done so far uses a 1-ml whole blood specimen.

As I alluded to on the early slide, we have designed the assay to detect multiple species of *Babesia*. We have actually tested four of the major species: *microti, duncani*, *divergens,* and *venatorum*. As you can see, this is an analytical study, where we did serial dilutions of *in vitro* synthesized transcripts of these four targets. The sensitivity appears to be pretty good at this stage for most -- here is *microti* on the left, *duncani*, *divergens*, *venatorum*. In most cases we are able to detect at 30 copies. The *divergens* was not quite as sensitive. That is something that shouldn’t be a challenge to correct. The *venatorum* also seemed very sensitive.

I would like to introduce the concept of why we think pooled testing could way, based on the approach that we are taking with this assay. I am showing an example here of ten parasites present in 1 ml. We add that to our lysis solution and we release maybe 2,500 copies of the target per ml. If we assay 500 microliters, there is a high probability that that would be reactive, based on the typical sensitivity of these types of tests.

Testing in pools would be done differently than the way pools are tested today. Today the plasma samples are combined, equal aliquots, and then tested in various pool sizes. In this case the pooling would be done on the pre-lysed samples. So the RNA would be released prior to pooling. There would be a large number of copies, based on the multi-copy target that we are detecting. In this particular example we are showing 156 copies per ml. Five hundred microliters of that would be assayed. There is a pretty good probability -- I put a question mark next to “reactive” -- a pretty good probability that that would also be reactive, in a relatively large pool size.

To illustrate this, we did a model experiment using hamster infected specimens that we received from Laura Tinetti and David Leiby when he was with the American Red Cross. This is showing a serial dilution of infected hamster blood -- in human blood in one case and then infected hamster blood in the lysis solution. The reason we diluted in human whole blood is that we wanted to keep the red blood cells and the parasite intact as we diluted. From this experiment, you can see at around five parasites per ml, we reach the limit of detection. After that, the parasite simply is diluted out. After that, we started doing threefold dilutions. However, when the RNA is released first, you can continue detecting 3, 9, 27 full dilutions after that, still at 100 percent, effectively detecting .18 of a parasite, so a fraction of a parasite, based on the multi-copy target.

Based on this kind of experiment, we thought that pooling might be effective. There are obvious advantages to testing more of the at-risk states with an approach like this.

To test this out in something close to clinical specimens, we obtained 24 red blood cell specimens from the American Red Cross. These had all been shown to be infectious by hamster injection. These red blood cell specimens were stored in Adsol, so there was a little bit of a dilution factor that when we did the experiment, we did not account for.

In this particular case, we tested 500 microliters of the RBC specimen, added it to the lysis reagent, as I showed in the previous slides, and then tested 500 microliters of that on the Panther System. For the pooled testing, we simply pooled the lysed material, prepared exactly the same way as the IDT samples, and then diluted that in lysed human whole blood to simulate pools of 4, 8, and 16 donation pools.

Here are the results. On the right, in green, are the results with the TMA assay. You can see, in the individual donor format, 100 percent were detected, so all 24. We didn’t test the entire 24 at a 1-to-4 simulated pool, but 100 percent of the ones that we tested there were also reactive. We failed to detect one sample in the 1-to-8 dilution and then in the 1-to-16, two. If you compare to the reference result, the research PCR result, the pool of

16 in this test -- and this is a limited sample set -- was as sensitive, 91.7 percent versus 91.7 percent. So we definitely found that these results were encouraging. We definitely think that the amount of testing could increase if pooled testing were to occur.

That’s where we are in terms of data as of today. I just want to summarize very quickly.

We have shown the feasibility of a new sample prep method for intraerythrocytic parasites. We have developed what we consider a sensitive prototype NAT assay for the detection of *Babesia* on a fully automated system. What I think is really important is that we can detect all four clinically relevant species of *Babesia*. We have demonstrated, in a very preliminary sense, the effectiveness of pooled testing. As I mentioned, pools of 16 lysed donations showed encouraging preliminary sensitivity results, comparable to the current research PCR assay. Further optimization of the assay system is under way.

Thank you very much for the opportunity to address the FDA and the committee.

DR. JACKSON: Thank you very much, Dr. Linnen.

Next we have Dr. David Pombo, from Cape Cod Hospital.

DR. POMBO: Thank you. My name is David Pombo. I’m the medical director for infection prevention at Cape Cod Healthcare and also an infectious disease doctor.

I was interested in presenting some clinical cases I have seen, all within the last two months, of *Babesia*, two of which I believe are transfusion-related and one which kind of illustrates some of the concepts which were discussed here by the previous speakers.

Just for the record, I have no relevant financial disclosures with any companies related to this meeting today.

Cape Cod Healthcare is a small system of the two hospitals that exist on Cape Cod. One is in Hyannis. It’s called Cap Cod Hospital. The other is in Falmouth and is called Falmouth Hospital. Cape Cod is the larger of the two. It’s a median-size hospital. Together, these comprise about 350 beds.

Just for background, we have a blood collection center at Cape Cod Hospital. It collects 4,500 donations per year, which are then tested at Rhode Island Blood Bank. Because the need is greater than the 4,500 units per year, we purchase the additional 7,000 units that are -- the difference between the 7,000 units that are transfused there and the 500 units at Falmouth Hospital. Everything that is collected on Cape Cod comes back to these two hospitals.

The cases I would like to go over briefly today:

• The first is a confirmed transfusion-transmitted babesiosis case, which I saw in early March.

• The second is a transfusion-associated babesiosis case, which I thought was likely, but found out only a few days ago that it was not confirmed. But I think it is a possible case.

• The third is a fellow I saw just two weeks ago who presents the risk. I am just going to put him up for your consideration, what we deal with.

The history on case 1 is a 73-year-old male who presented last fall with B-cell non-Hodgkin's lymphoma of his rib, presented with pain, and was treated with rib resection and R-CHOP chemotherapy every four weeks. The "R" is Rituxan. He had never received transfusions prior to November 2014, at which point he received four units of packed red blood cells on those two dates. He did well, until Christmas, when he was seen in the emergency department with fever and hypotension. He had positive smears by microscopy, with a parasitemia of about 1 percent. He was admitted. Interestingly, the hospital doctors did not consult infectious disease, and he was treated and discharged with azithromycin and atovaquone for ten days, and was clinically improved.

He continued with chemotherapy. On routine CBC in early March, he had detected parasitemias on a screening smear. No fever was documented. He was referred to our clinic. He now is being re-treated with long-term azithromycin/atovaquone/doxycycline through the time of his chemotherapy.

The reason I present this case is that he is probably about the third patient I have seen with relapse who has been treated with Rituxan. I believe it has been previously reported that Rituxan is a risk factor for these patients relapsing. I think that is interesting.

The transfusion history is here. No previous transfusions. He received the four units in November and then two units in January, three units in February, and one in March. Testing of one unit from the Rhode Island Blood Center for the November transfusions was positive for *B. microti* by IFA and PCR. Three other units tested negative -- actually, two other units tested negative. One of the four from November was actually collected at Cape Cod Hospital. This was likely his exposure. I will call that a confirmed case.

Case number 2: The history is an 82-year-old male with stage IV diffused B-cell non-Hodgkin’s lymphoma which presented as a diffuse lymphadenopathy of the internal organs in July of 2013. He also actually was diagnosed two or three weeks earlier with hemolytic anemia, which was felt to be related to the non-Hodgkin’s lymphoma. He also was treated with R-CHOP chemotherapy and was in remission for approximately one year. Since that remission about July of 2014, he has been continued on Rituxan and IVIG every eight weeks. He also received two units of packed red blood cells in March, March 12 of 2015 and March 20 of 2015.

He did well and actually felt well until he presented to the emergency department on April 8 with severe anemia. He had dropped his hemoglobin from approximately 12 to 7½. He was hypotensive and he had 5.5 percent parasitemia. He had a rough course in the ICU, very unstable. He was treated with antibiotics for ten days, slowly improved. Parasites cleared. He survived and was discharged home. He is currently on long-term azithromycin and atovaquone, given his need for continued Rituxan therapy. It’s not clear what we are going to do with him.

His transfusion history: He has been extensively transfused. He has had 34 packed red cell units, going back to August of 2013 through March of 2015, after which he became ill. The two units on March 12 and two units on March 20 all came from the Rhode Island Blood Center. Prior to that, his previous transfusion was back in November, and then prior to that in August. We were able to find and test the five most recent units from the Rhode Island Blood Center. All were negative by IFA and PCR.

I suppose they probably were tested by your methodology at IMUGEN. I’m not sure of that. I don't have documentation of that. Speaking with this gentleman, he has had no recent outdoor exposure. The last time he was out in the woods was in July of 2014. He has no pets. He had no known tick bites. He was actually hospitalized with other medical complications most of the winter. With the winter we had on Cape Cod, he wasn’t really out at all. No other earlier units were available for testing. I thought this was an interesting case. I would say it’s a possible transfusion-associated case. We can’t know for sure.

The third case is just a fellow referred to our clinic. He is 60 years old. He has a long history of low back pain, fatigue, and malaise, since 2012. He saw his doctor for that in April. He has had no fevers, no sweats, no dyspnea. He has been gaining weight. He had a history of a tick bite in 2010. He came in complaining of fatigue, some low back radiating through his thighs bilaterally, not worse. The concern was for rheumatoid arthritis. He had extensive normal routine laboratory testing, normal white count, normal platelets, normal differential, normal sed rate, normal kidney. Urinalysis was normal. The doctor didn’t really indicate that there was any concern about tick-borne illness, but he checked the boxes anyway and referred him to a rheumatologist. The history was that he retired last year and spent time outdoors in the fall.

The previous Lyme serology we had on record from 2009 was documented negative at IMUGEN. He reported one at Quest in 2011 that was negative as well, but I didn’t have documentation of that. In April of this year, he had *Anaplasma* PCR-negative, *Babesia* PCR-positive. No *Babesia* serology was ordered. I didn’t have that available. He had a strongly positive IgG reaction on the EIA, negative M and A. I think this is a fully developed Lyme serology. Probably the PCR for the *Babesia* is something that has been there for a number of months, probably five or six months.

Fortunately, the patient denied donating blood in the past. He was treated with a ten-day course of azithro. He represents that even with good clinical screening, and even some testing, you can’t pick these people up. He just illustrates the issues we are dealing with.

I thought it was interesting that we have a relatively small hospital where I work, but within a short period of time, we have had these two cases. I think they are probably really both -- the first two cases are probably both related to transfusion, and it’s not even the busy time yet. I just thought that was interesting.

Thank you very much.

DR. JACKSON: Thank you very much.

Next, Dr. Louis Katz, from the American Blood

Centers.

DR. KATZ: Hi. I’m Dr. Louis Katz, chief medical officer of America’s Blood Centers, ID physician.

I have no relationship to any of the companies making tests, but have taken funds for a variety of activities from Cerus and Terumo, who are working on pathogen-reduction technologies, noticeably absent -- probably appropriate -- this year from this set of discussions.

Thanks for the opportunity to address the committee. We are the association of independent FDA-licensed blood centers, responsible for half the US blood supply. We appreciate the opportunity to present our thoughts.

The risk of transfusion recipient infection from *B. microti* red cells in highly endemic areas is higher than that which we tolerate for the classic transfusion-transmitted infections, HIV, HBV, and HCV, as Dr. Stramer showed you, and justifies consideration by the BPAC and, we believe, mitigation interventions. Assays in development appear to have suitable performance characteristics for donor screening, based on published data, data presented in a variety of public forums, including this meeting.

The levels of attributable morbidity and mortality from transfusion-transmitted babesiosis are difficult to assess in the current literature. Some of our members have questioned whether it rises to the level of requiring a donor-testing intervention.

Regardless of that discussion, the risk-mitigation strategy that evolves must include effective education of physicians responsible for transfusion about the risks from *B. microti*. Their awareness will allow appropriate clinical suspicion of the diagnosis, facilitating accurate diagnosis, effective treatment, and, importantly, complete public health reporting for ongoing surveillance regarding the geographic extent of transfusion transmission.

B. microti infection is currently so geographically heterogeneous that any universal recommendation in the US represents, in our opinion, an inappropriate allocation of scarce resources in pursuit of, quote, zero risk. It is key, then, that policy development for this infection be the subject of detailed health economic analysis. We support a recommendation for regional testing, but its eventual scope, including both the geographic extent and the exact test algorithm, should be determined considering all appropriate evidence using agreed-upon risk-tolerance thresholds. Multiple stakeholders, including those from outside the blood community-FDA axis, must be engaged in the decision-making exercise.

ABC objects to the agency’s reliance on unvalidated diagnostic data from the CMS claims database in the FDA modeling referenced in the issue summary, current discussion in today’s presentations. The accuracy of the diagnoses has not been established and extrapolating the policy alternatives from such data, which is demographically not representative of the database, is probably inappropriate at this point.

Under the prospective payment systems that dominate US health care, especially DRGs, the increasing cost of blood safety is never reimbursed in a timely fashion. FDA is prohibited from considering cost issues and does not or cannot influence CMS and other third-party payers in a timely fashion, based on recent history. We maintain that this disconnect is inimical to sound health-care policy development and implementation, most particularly in the unprecedented circumstance of a geographically restricted infection like babesiosis. Our members have expressed grave concerns that regional testing has the potential to financially destabilize centers that collect in endemic states because hospitals are free to contract with suppliers outside endemic states for an untested inventory at a lower price.

There are numerous contextual issues to consider surrounding the implementation of new blood safety interventions. A substantial number of blood centers are operating at unsustainable margins already. Revenues have declined due to the penetration into medical practice by clinically appropriate, conservative transfusion practices. Hospitals then perceive additional costs for blood as a direct negative impact on their margins, and the subsequent competition among collection organizations for hospital and hospital system business based on price further erodes revenues.

These circumstances fail to recognize the unique status of blood and blood community infrastructure in the provision of medical care -- what we call the insurance value of replete blood bank inventories that support both routine and urgent surgical and medical interventions. In this view, the robust availability of blood in the hospital blood bank, regardless of whether it is actually transfused or not, permits timely responses to day-to-day variations in blood needs within the health-care system. The blood community's ability to maintain the infrastructure needed for surge capacity, safety improvements like *B. microti* testing, pathogen reduction, and value-added services that range from immunohematology reference laboratories to patient blood-management expertise is threatened.

A requirement for *B. microti* testing must accommodate these contextual issues. That requires a broad stakeholder engagement exercise that FDA and BPAC are not appropriately constituted or charged to undertake. The decisional process should be a full risk-based decision-making exercise that prominently includes explicit discussions of tolerable risk. It must be conducted outside our usual blood community-BPAC-FDA silo. A minimal list of the important stakeholders includes payers -- that includes CMS -- hospital administrators, transfusion clinicians outside of transfusion medicine, patient advocates, bioethicists, and health-care economists. The HHS Advisory Committee on Blood and Tissue Safety and Availability is best positioned to address these issues and is charged with the responsibility to undertake these kinds of exercises. FDA should request that the committee consider this activity before promulgating guidance, regardless of whether the agency in the very near future approves one or more of the assays under development.

In this context -- that is, a comprehensive risk-based decision-making process undertaken from the societal perspective -- ABC is committed to doing the right thing both for patient safety and a sustainable, robust blood supply.

In response to the specific questions asked, if testing is recommended, regional, year-round testing is preferred by most of our members. This is based both on the occurrence of clinical babesiosis and identification of infected and infectious donors across nearly all months, as you have heard today. We do not believe that nationwide testing is appropriate with either or both assays at this time, but articulating an appropriate testing algorithm is dependent on understanding what, if any, risk is tolerable.

Again, until tolerable risk is understood, the second question is difficult to answer. Unpublished health economic analyses that Sue Stramer has shown you some of today suggest that the most effective strategy in highly endemic states may be both nucleic acid and antibody testing, and that dual testing can meet rational standards of cost-effectiveness.

Regarding reentry, since the donor will be required to be *B. microti* test-negative to be reentered, a negative result on the test of record at an interval long enough for true positives to wane is appropriate. One or two years would be acceptable, with the understanding that donors can be reevaluated serially until qualified.

Thank you.

DR. JACKSON: Thank you very much.

We are running behind. This was our last speaker who asked to be able to speak at the open public hearing session. We are going to take a ten-minute break now and then come back for about a 50-minute discussion on the issue.

(Brief recess)

Agenda Item: Open Committee Discussion

DR. JACKSON: As you have heard, we have been asked to consider three questions. We will tailor our discussion question by question by question, and we will vote after each question. We do have some new technology here, these little turning point things. I don't know how to work them yet, but we will, I’m sure, find out when we get to that point.

Do you want to say something at this point?

DR. KUMAR: Good afternoon. I’m going to read you the questions:

1. Do the available scientific data and FDA analysis support the concept of nationwide, year-round testing of blood donations for babesiosis risk by an antibody-based test?

Before I read the option here, I think, Dr. Jackson, you would like to lead the discussion here.

DR. JACKSON: Yes, that will be fine.

I think we have heard from a number of speakers today and have seen the data from CDC and the FDA, as well as Dr. Stramer’s data where they have been doing their selective testing in endemic and non-endemic regions.

People’s perspectives, thoughts on this issue, for antibody testing?

DR. SIMON: As you heard, the industry that I represent is somewhat conflicted about this. Obviously the issue of regional testing and the impact on the centers in those regions and all the economic impacts have been brought to your attention. Certainly we hope that this will move on to that kind of analysis before the Safety and Availability Committee.

But with regard to the specific question, based on the data we have heard, particularly impressive information from Dr. Stramer on the difference in the risks, I would hope that we would not support nationwide testing and would, instead, look for regional testing.

DR. SCHEXNEIDER: While I sincerely appreciated Dr. Katz’s comments and commitment to regional testing, I am persuaded by the mobility and travel that people have in our country and would be concerned that if we had regional testing, we would miss people who traveled to endemic areas and then came back to their home states to donate. For that reason primarily, I’m supportive of nationwide antibody testing.

DR. SANDBERG: I appreciate Dr. Stramer’s concerns and some of the other ones that were mentioned about the FDA model, but I think it’s a really high-quality piece of work and I think that they did address some of the issues, particularly concerning the CMS data. By comparing it to the CDC data, I think that adds strength to their analysis.

When you look at their results, you can see that there is, of course, a tradeoff between the number of positive units that you are obtaining and the number of false positives that you are losing the process. I think when you look at carefully at their results, it really doesn’t support having nationwide testing, because you don't gain very much in terms of finding positive units, but you do lose quite a bit with false-positive results.

DR. RAGNI: I guess I want to say a few things. I am confounded by being in Pennsylvania. But I would also say that one of the really critical things that I think we need to think about is the group of folks who are receiving the blood. The largest group is hematologic. Half of them have sickle cell disease. They are autosplenectomized. They are not just located in endemic areas. I have some concerns.

In addition, the most likely reason to have a transfusion is an elderly patient with some sort of problem, such as the lymphomas that we heard about.

I think we have the tools, as so beautifully described by many of our speakers, to make a safer blood supply. I think this is very different than the risks of

HIV, Hep B, Hep C, all these other things. So I am very concerned that we have patient populations at major risk if we do not adopt this as a nationwide, year-round approach.

DR. STOWELL: I, on the other hand, would support also a regional approach rather than a nationwide approach. I think we approach sort of a situation of diminishing returns for expenditures in terms of the testing and effort that is put into it. We have seen that something like 95 percent of the cases were associated with the endemic regions.

I think one caveat is that we have to keep a little bit ahead of the curve. This epidemic is going to presumably follow the path of Lyme disease, and where the mice go and spread Lyme, they will be spreading *Babesia* as well. So whatever we decide in terms of what the appropriate region is, there has to be a mechanism in place so that we can amend that as we go forward.

It would be easy to say, well, let’s test everybody for everything. That is an easy answer. The reality that we work in in health care is that we just really can’t keep taking this kind of approach.

DR. DEMARIA: My perspective on this obviously is from a public health standpoint. There is no question that if Massachusetts was a standalone country with a unified regulatory and blood collection system, we would have been screening a long time ago, with the kinds of double-digit increases and incidence that we are seeing just in our surveillance data and the numbers of transfusion-transmitted cases. But the difficulty is it is so geographically heterogeneous. It makes absolute sense to me to screen in Massachusetts, but if I was in Wyoming or Arizona or someplace else where the numbers of cases, even with people traveling, are so limited -- in public health we are always balancing the benefits and the risks of doing things. I think that the overall risk in the country doesn’t justify universal screening.

I don't know what the right number is. Five seems too low in terms of states, and 15 is getting up on the high side. Maybe nine is the number. But I worry about Pennsylvania and Maryland and Virginia. But I don't think it justifies universal, nationwide screening.

DR. JACKSON: Now, we are talking about just antibody on this question.

DR. DEMARIA: Right.

DR. JACKSON: Dr. Maguire.

DR. MAGUIRE: I just worry about the number of cases that would be not prevented without universal screening. This is an event that leads to fatalities. We have talked about the increased risk of transmission because of the vector, but I think there is also an increased risk of bad outcomes with the changing nature of the recipient population. I think the example we heard about of Rituximab -- the new biologic agents are being used in much, much greater numbers of patients. So I think the pool of even younger persons who are at risk for bad outcomes is increasing as well.

DR. NELSON: It’s a difficult question. Not only do patients move, but blood moves. I think clearly the question of whether or not to screen with nucleic acid testing in the high-risk areas is an easy question to answer. The question of universal blood tests of all donors is more difficult.

But I am sort of intending to suggest that it may be the possibility. We don't yet know how many cases have been missed. In areas where there isn’t a high suspicion, they may not even be recognized. That worries me.

I suppose if universal antibody screening was done and it was found not to be useful, it could be dropped. We rarely drop screening tests, but I think it would be a possibility.

So I’m sort of torn between whether it should be implemented now and then evaluated -- that is one other option. It could be tried for a year and see what happens. We would then have much better nationwide data, which we don't -- we have some data that is not regional, but more data would be useful. I don't know anything about how much this would cost, but that’s not what we are supposed to discuss, I guess.

DR. LEITMAN: I usually have strong feelings on this, and I have difficulty having a strong feeling on this. I think I am closest to what Kenrad just expressed. In 30 years of blood banking, it has been hammered into us to use this precautionary principle and do what we can for incremental improvements in blood safety, even if they are just incremental. Now we are hearing that there are substantial data, epidemiologic, and cost reasons not to implement universal testing.

There is something we haven’t thought of. There is something about donors who love donating. It’s part of their lifestyle. If you tell a donor they can’t donate because they have a serologic-positive and it might be a false positive, there are going to be a fair number of donors who are going to go to their physicians and get repeated testing and perhaps get treatment that doesn’t need to be given to them, and want to come back, and pester blood centers. The wider the net you spread for testing, the larger the number of such donors there will be. So that is a negative against nationwide serologic testing.

But animals move and mice move and blood moves. Our cases were in Maryland. So I am influenced by that. I am influenced by what Dr. Katz commented on, the financial disconnect. There are going to be more expensive units because there is more testing in certain regions of the country, difficulty in moving those units around outside of that region.

There are lots of complex issues.

I am very glad that something is going to be implemented, that something will be implemented in states. I’m happy with either nine or 15 with what is implemented, although we are not being asked that right now. But I really find it very difficult to make a recommendation on universal serologic versus regional high-risk serologic.

DR. BONILLA: I think everyone around the table supports at least regional testing. We have heard lots of arguments, pro and con, about benefits and risks of universal, nationwide testing. But you also have to look at the way the question is written. Your answer then depends on how you interpret the phrase “support the concept.” The information presented today certainly supports the idea that there is risk reduction associated with broader screening. Whether that is too much risk reduction I think is open to debate. Certainly we can get to the same residual risk of HIV and hepatitis if we institute regional screening. But then do we need to go that extra mile for that last incremental step nationwide? I don't know.

But if I broadly interpret the phrase “support the concept of nationwide, year-round testing,” then I think we have to say, yes, the evidence supports the concept. Whether you choose to go that far I think depends on other considerations.

DR. BAKER: I wish that the data presented had some ethnic breakdowns. When I look at sickle cell disease and the infant mortality, which is higher among African-Americans -- and sickle cell disease also has a Hispanic population that is affected. That is rarely recognized. That would have helped with decision making.

DR. LERNER: I would also like to again underscore the fact that more and more patients with hemoglobinopathies, the kinds of patients you are talking about, are being transfused for a variety of new indications. I think we do really need to think about the people who are receiving these transfusions.

DR. JACKSON: My opinion on this: I’m in favor of this, for several of the reasons mentioned about the travel and that the epidemic is likely to expand -- mice don't walk as fast as birds fly, but I’m sure it is going to expand -- also the idea of just keeping it simple. I can see that if I am in New York and I need blood and something comes across the border from Pennsylvania, that is not tested, yet it’s one mile across the river. It starts getting complicated.

The types of patients that are susceptible are quite broad. It’s not like CMV, where you tend to focus maybe on just transplant recipients, like we used to do before we had all the leukoreduction. It seems everybody we plasma-exchange these days is on Rituximab, and it seems to be growing more and more.

I know we are not supposed to talk about cost, but antibody testing is typically not that expensive, and we are using less blood. From the hospital’s perspective, we are actually being more cost-effective, in some ways, than ever before. And it’s potentially a fatal disease. So I think it’s worth doing.

Any other comments on this? Then we’ll vote.

LCDR EMERY: Basically, all three of the questions that we have to vote on -- I believe we will be using the 1, 2, 3, which is the top two buttons.

DR. JACKSON: Does everybody understand that? Yes is 1, no is 2, abstain is 3.

(Technical problem with voting system)

LCDR EMERY: Because you voted, I will be able to see each individual vote, and the ones that I am able to read I will mark for the record. Then the ones that did not get read, I will ask you for your vote, and we will have it in the public record.

(The vote was taken.)

Dr. Maguire is yes. Dr. DeMaria is no. Dr. Stowell is no. Dr. Ragni is yes. Dr. Leitman is yes. Dr. Durkalski is yes. Dr. Basavaraju is yes. Dr. Jackson is yes. Dr. Bonilla is yes. Dr. Lerner is yes.

Dr. Schexneider did not read. Can I get your vote?

DR. SCHEXNEIDER: I voted yes.

LCDR EMERY: Dr. Schexneider is yes. Dr. Sandberg, it did not read yours.

DR. SANDBERG: No.

LCDR EMERY: Dr. Sandberg is no. Dr. Baker is yes. Dr. Nelson did not read. What is your vote, Dr. Nelson?

DR. NELSON: Yes.

LCDR EMERY: Dr. Nelson is yes.

How would the industry have voted on that?

DR. SIMON: I would have voted no.

DR. JACKSON: Okay, thank you.

We will move on to the second question.

DR. KUMAR: Does the committee agree that NAT-based testing should be performed in blood donations in certain high-risk states?

DR. JACKSON: Should we just deal with this question first?

DR. KUMAR: Yes.

DR. RAGNI: I would like to clarify, does this question also mean that we do not approve it to be done in non-endemic states? I want to understand what this question is asking us.

DR. KUMAR: The way it was intended was to give you three options, five states, nine states, and 15 states plus Washington, DC.

Dr. Jackson, is it okay to proceed that way?

DR. JACKSON: I suppose we could. The fourth option there is, if you thought there was some alternative or not to do it, you could do that.

DR. KUMAR: But that was the option once we vote on these three options. But if none of those are preferred, then we are going to move on to discuss what the alternative options should be.

DR. JACKSON: But the question it shows here is, does the committee agree that this testing should be performed in high-risk states? If you don't think that at all, then we wouldn’t proceed to the second part.

DR. KUMAR: If you want to go that way, that’s okay.

DR. JACKSON: If you didn’t think we should do NAT testing at all, you wouldn’t consider these other options.

DR. SIMON: Based on the first vote, I would assume there is going to be support for NAT testing, and I think there is good data to support it in the high-risk states. I would go with the nine, as recommended by a couple of our speakers.

The facial expressions I'm seeing from across the table indicate that many people would like to see NAT done along with serology nationwide. I would just comment that we already have a very low yield in Wyoming and New Mexico and wherever with serology. Now we add NAT, which only has an incremental, slight pickup of a certain number of donations, window donations, that aren't serologically positive. If you do NAT nationwide, you would really get, I think, into a cost-ineffective -- I assume that's why the FDA implicitly assumed that we might want to do serology nationwide, but we probably wouldn't want to do NAT nationwide.

So I support their thinking and also support the industry presentations. I would suggest the nine-state model at the present time.

DR. JACKSON: But I think the first issue is, do people on this committee support NAT testing in high-risk states? I think that is probably likely, but we have to vote. Does anybody want to comment on that or do people want to just vote?

DR. STOWELL: One of the things I have been impressed with is that this is not a very tidy infection, like some of the viral infections we have been used to. There have been a significant number of window-period NAT-positives before seroconversion, where the NAT turns negative, then turns positive. There are some sensitivity issues, very long antibody tail-off periods, and so forth. It’s not neat and tidy.

I think it is very difficult to recommend doing antibody screening and not also do NAT testing. We have already answered the question that we are going to accept zero risk by saying we should do this everywhere. I don't see why we should not do the NAT testing. It’s a very, very small number we are going to pick up in Wyoming, but we have already said zero risk.

DR. JACKSON: You are suggesting that at least for high-risk states, we should --

DR. STOWELL: At least for high-risk states.

DR. JACKSON: So let’s address that issue first. Are there any other comments? Otherwise, we’ll just vote on that question that is up there right now: Does the committee agree that NAT-based testing should be performed in blood donations in certain high-risk states?

Please vote 1, 2, or 3.

(The vote was taken.)

LCDR EMERY: It appears we have 13.

Dr. Maguire is yes. Dr. DeMaria is yes. Dr.

Stowell is yes. Dr. Ragni is yes. Dr. Leitman is yes. Dr. Durkalski is yes. Dr. Basavaraju is yes. Dr. Jackson is yes. Dr. Bonilla is yes. Dr. Lerner is yes. Dr. Schexneider is yes.

Dr. Sandberg did not read. What is your vote, Dr. Sandberg?

DR. SANDBERG: Yes.

LCDR EMERY: Dr. Baker is yes. Dr. Nelson is yes.

DR. JACKSON: So that is unanimous.

We will go to the next questions.

DR. KUMAR: Since we already had the vote that NAT testing should be performed, we are presenting these three options for the committee to consider:

- First, the five states listed here: Connecticut, Massachusetts, Rhode Island, New York, and New Jersey.
- Nine states: Connecticut, Massachusetts, Rhode Island, New York, New Jersey, Minnesota, Wisconsin, New Hampshire, and Maine.
- Fifteen states plus Washington, DC: Connecticut, Massachusetts, Rhode Island, New York, New Jersey, Minnesota, Wisconsin, New Hampshire, Maine, Maryland, Washington, DC, Virginia, Vermont, Pennsylvania, Delaware, and the state of Florida.

DR. JACKSON: We open this up for comment on those options. There is, obviously, a fourth option, if you don't like any of these three.

DR. BASAVARAJU: I would think that the nine-state option isn’t -- it seems like it would exclude some areas that are probably high-endemic where the data may just not be that good or surveillance reporting is not as good. If you are going to include New York and New Hampshire, then you would logically include Vermont, because there is nothing geographically different about it, really, other than an arbitrary state boundary. We realize that Pennsylvania is a growing endemic area. So why would that not also include parts of Maryland? I would think the 15-state option would be the most logical way to go.

DR. JACKSON: Others?

DR. NELSON: On this one, it would seem like we should vote 1, 2, 3, or 4 rather than just go yes and no on all four of them, don't you think?

DR. JACKSON: That’s what we talked about, doing 1, 2, 3, 4.

DR. LEITMAN: Again, it’s hard to have very strong feelings about this. You are talking about adding NAT to serology, where serology picks up the vast majority of infected donors. You are adding mostly window period, and window period is most likely in most endemic areas. I don't feel pressed to enlarge the net to the 15 states, with reasonable reporting of hemovigilance and where serologically positive donors are occurring, and if it’s increasing in some states that are in that group 3 or in other places. The number of units that you could have interdicted that you didn’t is really tiny in terms of moving from 2 to 3. So I’m very comfortable with 2, with nine states.

DR. JACKSON: Other comments on this?

DR. DURKALSKI: I think that the numbers -- they are negligible differences. So in that sense, I would pick the 15 states based on the numbers and just the comments about potential spreading, making other states endemic. My only struggle is that I don't know what the implications are of spreading it to 15 states in the sense of implementation of it.

DR. MAGUIRE: For option 4, could we say something like greater than nine, less than 15?

DR. JACKSON: I think at this point, it’s just, none of the above, basically. It really is a “none of the above.”

DR. RAGNI: But isn’t it important to define what number 4 is? Is it all of them, none of them? I think it’s critical to distinguish the difference here.

DR. JACKSON: I don't know if we can suggest another alternative or not. It just says, please comment on that, as opposed to a vote. But I think the vote would be that you are just not in favor of one of the three above. Typically we don't come forward with new things to vote on.

Dr. Epstein?

DR. EPSTEIN: We focused on nine states that were known endemic. I think today we have heard a little bit of the discussion about Pennsylvania. Dr. Herwaldt informed us that CDC thinks there is, in fact, now an endemic focus in parts of eastern Pennsylvania. I would suggest that part B can be discussed independently, and if the sense of the committee is that certain states should be added to the nine, consider voting nine, as the minimum of those nine. Then, if the committee wishes to vote nine and then add particular additional states, we are certainly listening to that discussion. So it’s not a foreclosed issue if you vote the question as written.

DR. JACKSON: Other comments?

(No response)

Then we will vote. Again, I think on the 4, 4 is that you don't agree with any of the above. We will then talk about potential alternatives. Can we do that?

PARTICIPANT: I will put a fourth option in there real quick if you want me to.

LCDR EMERY: Is it all right to add a fourth option for “none of the above”?

DR. LEITMAN: I just heard Dr. Epstein say to vote for 1, 2, or 3, and then after the vote is taken, then discuss B, how you feel about your vote.

DR. JACKSON: If you don't agree with any of the three, then what do you do? Do you just abstain?

If you vote for one of those three, that’s fine, but if you don't like any of the three for some reason, then you abstain. I assume that’s the option.

DR. LERNER: But that abstention -- it’s a very heterogeneous --

DR. JACKSON: It is. That’s why then we comment on alternative options, I would think.

DR. DEMARIA: I think there has already been discussion about what FDA would or would not do in terms of expanding the area of NAT testing. If we are voting on this -- Dr. Ragni’s point -- if we are going to do universal serologic screening, then there is not much lost here, and we could vote for one of three of these, and it’s still going to be a process in evolution. I’m just imagining us voting state by state and whether that makes sense. I think you have to start somewhere, and these are three places to start. That is maybe what we should be voting on.

DR. JACKSON: We can do those three, then.

LCDR EMERY: To be clear, we are going to vote 1, 2, or 3, whether you would prefer 1, 2, or 3. Then we will discuss and comment on other options.

DR. JACKSON: Vote now.

(The vote was taken.)

LCDR EMERY: I will read the results into the official record. Dr. Maguire is 2. Dr. DeMaria is 2. Dr. Stowell is 3. Dr. Ragni is 3. Dr. Leitman is 2. Dr. Durkalski is 3. Dr. Basavaraju is 3. Dr. Jackson is 2. Dr. Bonilla is 2. Dr. Lerner is 3. Dr. Schexneider is 2. Dr. Sandberg is 2. Dr. Baker is 3. Dr. Nelson is 2.

DR. JACKSON: It’s 8 to 6, nine states.

Comments on alternative options?

DR. LEITMAN: We heard from Dr. Herwaldt that there appears to be an early endemic focus in the area of Pennsylvania that borders on New York, so it makes sense to add Pennsylvania in. I would ask the FDA to strongly consider that.

DR. JACKSON: Any other comments or states you want to add?

DR. NELSON: The serologic data would provide some epidemiologic basis for change, either dropping states that are in it or adding more. It seems to me that if there is more widespread serologic screening, you would have some data to make changes dynamically.

DR. SIMON: I think there should be mechanisms in place to add states as time goes on, as indicated based on the data. I'm comfortable with any of them, but I think the nine is a good starting point, plus or minus Pennsylvania.

DR. JACKSON: Any other comments before we go to the third question?

(No response)

The third question, please.

DR. KUMAR: For the third question, we are seeking comment on whether it would be appropriate to apply a time-based deferral for those donors who have *B. microti*-positive test results. If so, please advise on a suitable deferral period for donors who had *B. microti*-positive test results.

DR. JACKSON: I think we heard that some 80 percent were still antibody-positive after first testing positive. At the same time, we had some who were still NAT-positive and yet had not become antibody-positive. We heard some suggestions that at least a year or two would seem reasonable before coming back in and being tested again. But it would seem that, given the pathogenesis and how *Babesia* works, most people clear this and become antibody-negative. So it would seem that it wouldn't be an indefinite deferral, that you could come in and test again. That's just my opinion.

DR. LEITMAN: The data that we saw that was presented is on page 4 of document 14 of Dr. Stramer's slide show. Of those donors who had high titer, which is anything greater than 128, 80 percent remain positive, with a large number followed, at one year, even higher or the same at two years, and the numbers get very small, but still substantially positive at three and four years.

But then you look at the low titer. There was only one set of those, and the titer was 64. There are only eight subjects. It’s a little bit hard to comment on an N of 8. But that wasn’t an 80 percent seropositivity. That was a 40 percent. Of course, it suggests that if you were seropositive and it was a false-positive event, even though it was in duplicate, you are more likely to fall off at a year. I’m all in favor of reentering donors who are eliminated because of false-positive testing.

So that’s what you are faced with. We rarely implement reentry testing based on a titer or an OD of the repeatedly reactive test. You really can’t do that.

But I think one year is too short, and you will bring in a lot of donors who are going to be repeat-reactive and a lot of call-backs and a lot of business for the blood bank and busywork, for not much of a return. But I hate to defer people permanently. So two years or greater.

DR. JACKSON: Two years or greater is your suggestion.

DR. STOWELL: But I think a key thing also is that it can’t just be on the basis of time. It has to be tied to a reentry algorithm of some sort. So it is truly an indefinite deferral and then reentry contingent upon test results.

DR. JACKSON: I assume you would do both antibody and NAT testing again.

DR. NELSON: I think somebody who really tests positive -- this is an environmental reservoir, and if they are living in a place where their yard is full of ticks, *Ixodes* ticks, they are at risk of being reinfected, I would think. I don't think you could possibly reenter somebody whose antibody has disappeared, if you think it wasn’t a false positive, without reentering them and doing NAT testing as well, to be sure that they don't have a recent reinfection.

DR. LEITMAN: I didn’t mean to imply to just defer them without a reentry algorithm. I was trying to think of how long before you let them come back for that. I wanted to clarify that.

DR. JACKSON: Anyone else have comments on this?

(No response)

Okay, we were just asked to comment on this. There is no vote on this question.

All right, lunch.

LCDR EMERY: Please do not discuss any of the meeting topics while you are having lunch. All advisory committee discussions need to be made in public. Thank you.

(Recess for lunch)

AFTERNOON SESSION

Agenda Item: Committee Updates

DR. JACKSON: In the next half hour, we have two committee updates. These will be presented by Dr. Illoh, on considerations of hemoglobin S testing in blood donors, and then Alan Williams will be presenting the revised blood donor referral policy for men who have sex with men.

The FDA is not seeking advice or recommendations from the committee on this topic, but wanted you to hear the update on this and send your comments, if you have some, within the 60-day comment period. But it's not for discussion, really.

I think we can probably get started. Dr. Illoh, on hemoglobin S testing.

Agenda Item: Considerations for Hemoglobin S Testing in Blood Donors

DR. ILLOH: Good afternoon. My name is Orieji Illoh, and I will be just giving a summary of the recent discussion on hemoglobin S testing in blood donors that was held late last year with the Advisory Committee for Blood and Tissue Safety and Availability.

Just a little bit of background. Hemoglobin S results from a mutation in the beta gene -- basically you have a substitution of valine by glutamic acid -- and results in a change in the amino acid constitution of normal hemoglobin. Basically, you have resultant sickling.

As we all know, homozygous inheritance of hemoglobin S gene results in significant morbidity, which is typically addressed as sickle cell disease. Those who have heterozygous inheritance typically have clinically benign or asymptomatic state. It is typically called sickle cell trait. Even though these individuals live normal lives compared to people who do not have these mutations, it is known that they do have an increased risk of medical conditions. Rhabdomyolysis following extreme exertion in some young athletes and other medical conditions have been documented.

There are also potentially social implications of having the sickle cell trait -- for example, reproductive implications.

In the US population, about 100,000 individuals have sickle cell disease, while about 2.5 million have the sickle cell trait. Among those with sickle cell trait, about 1 in 12 individuals are African Americans. Studies show that about .8 percent of US blood donors have sickle cell trait. Sickle cell trait generally is safe for transfusion, and donations from African American donors are essential, especially for patients who need blood from such individuals. This includes sickle cell disease patients and other highly alloimmunized African American patients.

However, blood establishments do have occasions when they test for hemoglobin S. This could be part of dealing with a manufacturing process or selecting blood for specific patients that have specific clinical needs. I have on this table here situations for which hemoglobin S testing could be done or is currently done in blood establishments. It could be done at donation on a donor's sample so that they can select specific blood units for specific patients -- e.g., neonates or patients with sickle cell disease -- or it could be done to identify and divert units from the leukocyte-reduction process or glycerolization of red cells and freezing.

It could also be done after donation, for about the same reasons. Either you need to select units for specific patients, e.g., neonates or sickle cell disease patients -- typically this is done at a transfusion service level, so units are tested -- or it could also be done during the manufacture of blood products, e.g., for leukocyte-reduction failures.

In terms of testing methods, historically a lot of the testing method was performed using sickle solubility tests. These are screens that screen for hemoglobin S or other related hemoglobinopathies. It does not distinguish between sickle cell disease and sickle cell trait. It's just a screen. Typically this was done in select units or select donors to either make decisions on manufacturing or select specific units.

As you are all aware, molecular methods for red cell typing are being used increasingly. We recently approved the Immucor PreciseType test, which is used for red cell genotyping, but also includes a chip that can test for hemoglobin S. So basically any donor who is tested using this technology will automatically be tested for hemoglobin S.

At the meeting late last year, we asked the committee to comment on the ethical and logistic considerations in testing blood donors for hemoglobin S. We presented two main issues to them. One issue was the donor consent. Currently there are no FDA regulations or recommendations that specifically address hemoglobin S testing. FDA noted that the practice of informing donors for hemoglobin S testing varies and that the donor may not be informed if testing is performed to investigate manufacturing failures or to select a unit for transfusion.

We also presented the issue of donor notification and counseling. There are currently no FDA regulations or recommendations addressing notification of donors of hemoglobin S test results. The practice of donor notification/counseling varies among blood establishments, and a hospital transfusion service may not inform a blood collector of a hemoglobin S result that they may obtain when they test a unit of blood.

The ACBTSA presentations in order to deliberate on this topic included numerous presentations, including one from Dr. Lisa Lee. She basically summarized the Presidential Commission for the Study of Bioethical Issues, “The Ethical Implications of Incidental and Secondary Findings.” Then we had two speakers address ethical considerations as related to hemoglobin S testing among blood donors. I believe this was a balanced discussion between the two ethicists. Then we had discussions from independent blood centers, including Dr. Louis Katz and Dr. Ginzburg. Dr. Ginzburg discussed the perspectives from her blood center, New York Blood Center, while Dr. Katz presented a survey that was performed among member blood centers in America’s Blood Centers. Basically, his survey showed that the practice of informing donors, notifying or counseling donors varied among blood centers.

Then we got a transfusion service perspective from Dr. Naomi Luban, from Children’s Hospital here in DC, and then a patient/family perspective from Mr. Larry Allen, who is a parent of sickle cell patients.

The committee generated a couple of findings, which I will go over in the next two slides, and then provide recommendations.

Their findings included that there are limited, although well-documented donor health implications for sickle cell trait. They noted that knowledge of sickle cell trait status has significant psychological and social implications -- e.g., reproductive choice. Withholding of medically and socially sensitive information could undermine trust in the blood system.

They noted that donor notification and medical referral are the responsibility of the blood collection establishment, and they said counseling in regard to medical significance of sickle cell trait lies with the donor’s health-care provider. They also noted that while there may be potential adverse consequences of notification of test results, the overall benefits outweigh the risks.

They provided several recommendations. I will go over the major recommendations in the next two slides:

• They recommended to the Assistant Secretary of Health that donors should be informed that their donations may be tested for hemoglobin S and that they will be notified of positive results. They noted that donors who do not wish to be tested or notified may decline to donate.

• Secondly, donors who test positive for hemoglobin S or present with a known history of sickle cell trait should be encouraged to donate plasma or apheresis platelets as alternatives.

• They noted that opportunity should be provided for donors to become informed about the significance of sickle cell trait.

• They noted that transfusion services should inform blood collection establishments in instances where a product is found to be positive for hemoglobin S.

• Finally, they said additional research on testing blood donors for hemoglobin S should be performed, with dissemination of findings to clinicians and the public.

Those were the major recommendations from the committee.

So what next? FDA has reviewed and is still reviewing the recommendations from the committee and the presentations, and intends to develop draft guidance in consideration of the recommendations of the advisory committee. This draft guidance we intend to focus on their recommendations, including donor acknowledgment, where donors are informed about hemoglobin S and testing, donor notification -- so notification of donors of their hemoglobin S results, transfusion service notification of blood centers when they do test units and get positive results -- and, finally, addressing donor counseling, in terms of accuracy of hemoglobin S tests, recommending follow-up, and alternate products to donate.

This is the end of my summary.

DR. JACKSON: Thank you very much.

Like I said, this is an update. The draft guidance isn’t out yet?

DR. ILLOH: No.

DR. LEITMAN: Some blood centers are concerned that molecular testing for hemoglobin S is different than sickle cell screening testing, because it’s molecular and it’s germline, so it’s a different level of risk. Was that addressed in the discussions of the committee?

DR. ILLOH: It was not addressed at this meeting, but I believe the results of the molecular tests for Immucor specifically were addressed at the advisory committee, comparing data. They were comparable with the screen tests, with the -- I don't want to give wrong information. They were compared with, I think, probably electropheresis. The package insert, I believe, for Immucor labels it as a screen. What we are recommending is that if you do get a positive result as a donor, you still follow up with your physician to determine whether the results are appropriate.

DR. JACKSON: Thank you very much, Dr. Illoh.

Next, Dr. Alan Williams, on the considerations for revised blood donor deferral policy for men who have sex with men.

Agenda Item: Considerations for a Revised Blood Donor Deferral Policy for Men Who Have Sex with Men

DR. WILLIAMS: Thanks, Dr. Jackson. Good afternoon.

As many of you are aware, a draft guidance regarding reducing the risk of human immunodeficiency virus transmission by blood and blood products was posted on the CBER website yesterday. This included recommendations regarding blood donation deferral for men who have sex with men. Today’s update is to provide a brief background of some of the key events leading up to the development of this draft guidance and to provide specific instructions to the public on how to formally comment on a document within the next 60 days, which we strongly encourage.

I will also provide an update on the transfusion-transmissible infections monitoring system, which was discussed extensively at the December 2 meeting of this committee.

At this time, as Dr. Jackson mentioned, we are not seeking discussion or recommendation from the committee on these two topics.

I have a couple of high-level background slides. In September 1985, FDA recommended that blood establishments indefinitely defer male donors if they had sex with another man at least once since 1977. Following that, in April 1992, FDA issued a memorandum to blood establishments which reiterated the indefinite deferral for men who have sex with men, abbreviated here as MSM, and recommended indefinite deferral for commercial sex workers, intravenous drug users, and certain other individuals with an increased risk for HIV infection.

Since 1985, the risk of HIV transmission from blood transfusion has been reduced from approximately 1 in 2,500 units to a current estimated risk of approximately 1 in 1.47 million units. This is primarily due to, number one, deferral of donors identified by questionnaire as having an increased risk of transmitting HIV, improved donor education materials related to HIV risk and self-identification of HIV risk, and then, of course, advances in HIV testing of donated blood.

In June of 2010, the HHS Advisory Committee on Blood Safety and Availability, the ACBSA, recommended studies that might support a change in the MSM deferral policy. These studies were designed, funded, and implemented over the next four years. They included:

• First, a study funded by the NIH National Heart, Lung, and Blood Institute, entitled “REDS-II -- Transfusion-Transmitted Retrovirus and Hepatitis Rates and Risk Factors Study.” This program updated the epidemiology of transfusion-transmitted infections and risk factors in current blood donors, because national-level data related to this epidemiology had not been collected in about 15 years.

• Secondly, the CDC National Center for Health Statistics conducted cognitive studies on a donor history questionnaire. This was the second round of cognitive studies, and it produced some very useful and workable information from this latest set of interviews.

• Thirdly, in a program funded by both the Food and Drug Administration and the National Heart, Lung, and Blood Institute, an interview-based study called “Donation Rules Opinion Study,” or “BloodDROPS,” helped to assess levels of current noncompliance with current MSM deferral policy and get opinions of both the gay community and blood donors about future policy options.

The details of these studies were really elucidated in the November 2014 ACBTSA meeting. I refer you to that transcript for the data that were actually published. Many of these publications are now being prepared and should be available in the literature very soon.

As stated, in November 2014, the ACBTSA met to review the available evidence supporting a policy change and voted 16 to 2 to recommend a one-year deferral for men who have had sex with other men.

In December 2014, this committee discussed scientific considerations related to MSM deferral and strongly supported the establishment of a blood safety monitoring system. In that same month, in December, Commissioner Hamburg announced FDA's intent to recommend a policy change to the blood donor deferral period for MSM from an indefinite deferral to one year since last male-to-male sexual contact.

The draft guidance, entitled "Revised Recommendations for Reducing the Risk of Human Immunodeficiency Virus Transmission by Blood and Blood Products," was posted on the CBER website yesterday. The link is provided here, for those who have not yet located it and would like to. This draft guidance, when finalized, will implement FDA's recommendations for MSM blood donation policy and will supersede the longstanding 1992 memorandum, including deferral of other individuals with an increased risk for HIV infection. It also includes blood product management and labeling and donor testing for HIV.

There are quite a fair number of recommendations included. What I'm going to provide you with here is just an outline of the recommendations, together with a few comments.

First of all, the guidance recommends that blood centers, as they do now, provide materials to donors at every donation, but that these be updated with the elements described in this guidance.

Secondly, regarding donor deferral, the draft guidance updates the donor HIV risk elements and includes, specifically, deferral for 12 months from the most recent contact, a man who has had sex with another man during the past 12 months. Additionally, other HIV-related donor risk deferrals have been updated so that this guidance, when finalized, will supersede the 1992 FDA memorandum on the same subject.

Donor qualification: This section defines the criteria for subsequent donation eligibility for a donor who has been previously deferred for the elements specified in the guidance. There are also sections specific to testing requirements and considerations. For instance, this includes recommendations related to supplemental testing. It includes product retrieval and quarantine, notification of consignees, product disposition and labeling, and biologic product deviation reporting.

All draft guidance is really issued for comment purposes only. Those wishing to make comments -- again, we at FDA strongly encourage this -- can submit them electronically at www.regulations.gov., under the docket number listed here, FDA-2015-D-1211. Importantly, there is a 60-day comment period. So if you would like your comments to be definitely included in FDA's deliberations, get them in within the 60-day period. You can submit comments any time and they will be welcome, but to have them included in discussions related to finalization of the guidance, they should be in within the 60 days. For those who prefer to submit written comments, they can be submitted to the Division of Dockets. There is an address indicated in the cover sheet of the guidance. Under good guidance practices, development of final guidance will follow careful consideration of all the comments received in the 60-day comment period. We look forward to reviewing those.

Changing the topic a little bit, FDA recognizes the need to monitor blood safety and acknowledges the numerous recommendations from our advisory committees over the past decade to establish a sustained monitoring system for blood safety. There is now a prime opportunity to leverage the NHLBI REDS-II epidemiology study and develop a long-term representative blood safety monitoring program. This is being done by the Food and Drug Association, working with the National Heart, Lung, and Blood Institute. The program is designed to look at HIV, HBV, and HCV incidence, as well as prevalence and behavioral risk factors. Using the outcome measures that are available, there will be an interest in establishing predetermined alert levels to indicate the need for possible intervention -- as opposed to simply watching the data and trying to decide when it doesn't look right, to try to define action levels.

This program will provide ongoing data available to objectively assess changes in any blood safety initiatives. This would really apply to any screening-type initiatives, but, of course, would be applicable to the potential changes in donation by men who have had sex with men. It also establishes a framework for investigation of transfusion-transmissible emerging infectious diseases.

The program will have a strong laboratory component, which will include molecular characterization of identified HIV isolates from donors. It will include evaluation of donor HIV antibodies using assays capable of characterizing recent HIV infection, because the HIV antibody profile matures over time after initial infection. These recency tests may give an alternate measure of incidence or new infection.

The use of recency assays will be validated with pilot studies of stored donor samples and, as I mentioned, include the potential use of this set of assays to estimate HIV incidence among donors and increase the power of time-based trend analysis, such as before and after a policy change. This is to try to get past the expected low numbers of infections that would make it quite difficult from a power standpoint to produce statistically meaningful pre and post results.

In terms of structure, the FDA is particularly interested in this from a safety standpoint. One of our mandates is to provide oversight of our regulated products. It has taken a while to get a stable monitoring system in place, so we are looking forward to having this available. It will be a joint program, working with, as I mentioned, NHLBI, as well as with stakeholder agencies and with the blood community as a whole. The program will represent at least 50 percent of the US donor base -- so the greatest effort to the greatest extent possible to represent the national donor base. There is funding for a five-year contract, with annual task orders, which (a) provides sustained funding and (b) allows us to add additional scientifically relevant elements to the program, such as EID-related work, as the contract goes on.

There has been an active effort to do the contractual work to get this program funded and up and running. There was what’s known as a “Sources Sought” solicitation issued March 30 of this year. We had a robust response to that solicitation. Based on that information, there is currently the development of a request for proposals, which is expected to issue mid-June of 2015.

Following that, we expect the award of contracts and startup of the program.

Thank you very much.

DR. JACKSON: Thank you, Dr. Williams.

We are going to postpone the break and proceed with the review of the research programs in the Laboratory of Cellular Hematology. To start us off, we are going to get an overview of CBER research programs and OBRR research programs, starting with Dr. Monica Young, from CBER/FDA, on that.

Agenda Item: Topic II: Review of the Research Programs in the Laboratory of Cellular Hematology, Division of Hematology, OBRR

Overview of CBER Research Programs

DR. YOUNG: I'm going to start with an overview of the CBER research programs.

The CBER strategic goals really play a part in the CBER strategic plan. There are two documents that detail the CBER strategic goals: the CBER strategic plan and strategic plan for regulatory science and research. The CBER strategic goals include:

- Increase national preparedness.
- Improved global public health.
- Enhance the ability of science and technology to facilitate development of safe and effective biological

products.

- Ensure the safety of biological products.
- Advance regulatory science and research.
- Manage for organizational excellence.

One example of how the CBER research regulatory program really plays a part in advancing the public health can begin many times with a novel product. The novel product brings up several questions and regulatory challenges that regulatory science and research programs here in CBER use to discover new tools that will generate information to inform the regulatory policy and decision making and improve the data and benefit-risk assessments. The goal is to end up with licensed products that are available for the public health.

In CBER we have something called a researcher-regulator model. It accounts for about 20 percent of the CBER staff. In this model the researcher-regulator spends about 50 percent of their time performing the role of a full-time reviewer and approximately 50 percent of their time with their research program. The integration of research and review allows us to have relevance of our research, ensure that we have appropriate experts, the timeliness of regulatory review, and the usabilty of data that is generated.

CBER researchers collaborate with various government institutions and academic institutions, as well as industry and nonprofit organizations across the country. The data on this map was generated from the annual research reporting database from FY13.

Each research project is reviewed annually. The process begins with the principal investigator, who provides a progress report for each of their projects, also future plans and a budget request, presentations and other publications that they have generated throughout the year. This information is reviewed by the lab chief, the division director, the Office of the Associate Director for Research, and the Office of the Director. It’s reviewed for relevance, productivity, and quality. This information is turned into a research reporting database and is also used for allocation of funding.

Every four years, the principal investigator undergoes something called a cyclic peer review. We have external site visits, a panel of scientific experts who review the scientific research program for that principal investigator, who is a regulatory researcher. Then there is an internal review by the Promotion, Conversion, Evaluation Committee, the PCE Committee, which reviews, in addition to their research program, also their regulatory accomplishments.

There is a draft site visit report that is generated and distributed to the full advisory committee. The advisory committee gives the final approval for that report. This report is very important and is used in many ways in our center, first by the PEC Committee, which I have mentioned already, for personnel actions. It is also used by the PI for improving their research program, and it’s used by management for the allocation of resources when they are available.

I just want to mention briefly that last summer CBER relocated from the NIH campus to the White Oak campus. This move has provided us with several additional resources, including a state-of-the-art vivarium that includes an imaging facility for MRI, visional X-ray, IVIS, and ultrasound. Also there are expanded core facility technologies, including flow cytometry, confocal microscopy, and high-throughput sequencing and bioinformatics support. Additionally, there are ten biosafety level 3 suites available that are able to support the work on 12 infectious agents that are used by 36 principal investigators in CBER. Many of these suites also are able to house animals, and one suite is used for the sorting of live cells by confocal microscopy. There is also an insectarium for BSL-2 and BSL-3.

In addition, we have suites that are specifically designed for microarray and PCR, and we have an expanded

NMR facility, as well as a mass spec suite.

I have a few pictures just to share. This is from our core facility. We now have an Illumina HiSeq that is available for our researchers to use in their research programs, as well as the flow cytometry core facility that I mentioned. Here are some images from those different suites.

Also we have expanded mass spec, NMR, and confocal capacity. Because of our move, we are able to have a larger facility for each of these technologies.

With that, I'll take any questions. I want to thank the site visit committee and the advisory committee.

DR. JACKSON: Thank you very much, Dr. Young.

Any questions?

(No response)

We will move on to our next speaker, Dr. Atreya, who will be giving an overview of OBRR research programs.

Agenda Item: Overview of OBRR Research Programs

DR. ATREYA: Good afternoon, everybody.

I will be providing a brief overview of the Office of Blood Research and Review's research program.

The office mission is to ensure the safety, efficacy, and availability of blood products. This is achieved through:

- Regulation of blood and blood components, plasma derivatives, and analogous products.

- Blood donor screening tests and other medical devices, including software used to test, collect, process, or store donated blood.
- Retroviral diagnostic tests.

In order to fulfill our mission, we also have certain functions. Some of them are illustrated here:

- We establish policies and standards to assure donor safety and the safety, purity, and potency of blood and blood products.
- We review applications for investigational and commercial use of blood products, blood-related drugs and devices, and retroviral diagnostics.
- We perform establishment inspections and product investigations with other offices in the center and also in the FDA, and assist in regulatory compliance actions.
- We perform health hazard evaluations and risk assessments of blood and blood products.
- We engage in emergency preparedness -- for example, Ebola outbreak.
- We also do global outreach and cooperation programs.
- We organize scientific workshops on timely topics important to OBRR.

• We also conduct research to facilitate the development, manufacture, and evaluation of blood products and retroviral diagnostics.

On the left sidebar you can see that some of these functions are done by the reviewers and the whole chart is done by the researcher-reviewers, typically.

The vision for our research programs is to support the FDA’s initiatives in regulatory science, including medical countermeasures to facilitate product development through:

• Focus on scientific questions critical to effective regulation.

• We concentrate in the areas where our unique role as regulators is most contributory.

• We also have provision of an infrastructure for investigation of product limitations and failures.

• Advancing innovation in research areas that enrich FDA’s regulatory science base.

We have a variety of resources. Monica mentioned the high-level facilities and resources. In addition to that, our office has subject expertise, including general virology, retrovirology, bacteriology, parasitology, prions, cell biology, toxicology -- all the ingredients that we need to have for our reviewers, expertise in that. We have right now around 25 investigator -- that is, so-called researcher-reviewer -- initiated programs located in seven laboratories. Our programs are mostly funded by both internal FDA funds, as illustrated here -- some of them are Modernizing Science, medical countermeasure initiatives, Critical Path, Panflu, FDA Nanotechnology, Office of Women's Health, Office of Minority Health -- and external sources like NIAID, NIH, NHLBI, NCI, et cetera. We also have some cooperative research agreements, in addition to the office allocated operating budget for these research programs.

Our current research prorities: There are seven priorities. They are all equal, priority-wise.

• One of them is, as I listed here, advanced molecular and genetic characterization of blood-derived proteins, their recombinant and transgenic analogues.

• We develop methods to identify predictive markers of product quality, safety, and efficacy of *ex vivo* stored cellular blood components, including storage lesions and plasma derivatives.

• Safety evaluation of *ex vivo* stored blood components and other blood products with respect to pathogen contamination and inactivation.

• Adaptation and evaluation of emerging and new technologies for high-sensitivity detection and novel reduction of infectious agents in blood and blood products.

• Preparedness and risk management with respect to emerging, reemerging, and terrorism-related, as well as neglected tropical pathogens with regards to blood safety and availability.

• Development and evaluation of reference materials, standards, and assays for infectious agents and HLA genotyping for product assessment and lot release testing towards modernizing the FDA science base and enhancing international collaboration.

• Evaluation of the impact of genetic polymorphisms in recipients to facilitate application of personalized medicine to the development and delivery of certain blood products.

Last year -- I am briefly mentioning here the research accomplishments. Our office has published over 90 publications in peer-reviewed journals. We have received $5.5 million of intramural funding and $1.8 million of funding from outside FDA and $1 million of funding through CRADAs. We had five cooperative research agreements established last year. Those funds were able to support 63 contract research staff through these funding mechanisms.

The office also has some leveraging efforts with the NIH. Some of them are listed here. We have some collaborative work going on with the NIH Clinical Center, NHLBI, NIAID, NCBI, and NCI, as illustrated here.

OBRR, our office, also participates in global outreach. The office members are participants in many WHO efforts -- for example, the WHO Collaborating Center for Biological Standardization, the Expert Committee on Biological Standardization, Blood Regulators Network, prequalification programs for diagnostics. Some of the members in the office are also observers for the European Directorate for the Quality of Medicines and Healthcare, Blood Transfusion Sector, and members in the International Society of Blood Transfusion Working Groups on Transfusion-Transmitted Diseases, hemovigilance, and global blood safety. It also participates in FDA/EMA/Health Canada Blood Cluster as part of our global outreach.

In conclusion, we believe that the research is integral to the mission of OBRR and CBER and FDA. Our research office for such programs facilitates product evaluation and development, where feasible, and is aligned with the regulatory science mission of FDA.

Thank you.

DR. JACKSON: Thank you very much, Dr. Atreya.

Any questions for him on this topic?

(No response)

We will go to Dr. Basil Golding, who will give an overview of the Division of Hematology research program.

Agenda Item: Overview of the Division of

Hematology Research Program

DR. GOLDING: Good afternoon. My job is to tell you about the Division of Hematology, and in particular to try and explain to you in ten minutes how our research program is related to our mission at the FDA.

The division consists of four laboratories or research units, listed here: the Laboratory of Biochemistry and Vascular Biology, Laboratory of Cellular Hematology, Laboratory of Hemostasis, and the Laboratory of Plasma Derivatives. In each laboratory there are principal investigators, three in this laboratory, three in the Laboratory of Cellular Hematology that you are reviewing today, four in the Laboratory of Hemostasis, and four in the Laboratory of Plasma Derivatives, with a total of 14 principal investigators.

The Laboratory of Biochemistry and Vascular Biology is primarily involved, in terms of regulation, with hemoglobin-based oxygen carriers, albumin, and volume expanders. They also regulate C1 esterase inhibitor for hereditary angioedema. The Laboratory of Cellular Hematology regulates blood components, plasma, platelets, red cells. The Laboratory of Hemostasis regulates all the coagulation products, procoagulant, anticoagulant. The Laboratory of Plasma Derivatives regulates immune globulin, intravenous polyclonal. They also regulate alpha-1 proteinase inhibitor, which is used as replacement therapy.

The scope of regulation and research is very diverse, as you can understand from the different products. The basic idea is that research should help to solve regulatory problems. This is the basis for the Critical Path initiative at the FDA. This serves to enhance the expertise of scientific investigators who have review responsibility for these products.

The scientific evaluation of biologic products derived from blood includes those isolated from blood as components -- as I have already mentioned, red cells, platelets, and plasma -- and also those products that are purified from the plasma as proteins, and, in addition, analogous materials manufactured by recombinant DNA technology, including products that are made from transgenic animals. I think in the FDA we were the first division to approve a product made in a transgenic animal, and that was antithrombin III.

Clinical applications of these products are also very diverse. They include blood transfusions, prophylaxis and/or treatment of bleeding disorders with clotting factors, treatment of trauma with volume expanders, hemoglobin substitutes, and, obviously, blood components, and treatment of infectious diseases -- a whole variety of infectious diseases that are treated by treating primary immune deficiency patients with the polyclonal immune globulins, but also specific conditions, such as hepatitis B, rabies, tetanus, and many others. The treatment also includes treatment of toxins and antivenoms for snake bites. The immunological deficits and autoimmune disorders are indications for the immune globulins and replacement therapies using congenital or acquired deficiencies such as I have already mentioned, C1 esterase inhibitor and alpha-1 proteinase inhibitor.

The products that we regulate, and the process, I will describe to some extent on this slide. The applications and products, as you hopefully have understood to this point, include the whole gamut of what FDA regulates. This includes biologics, drugs, and devices. The products themselves are very complex, including the complex proteins and carbohydrate polymers.

The evaluation process of these products includes scientific review, laboratory investigation if needed, development of lot-release tests, which involve development of standards and assays for lot release of different products. This work is often done in collaboration with international and other national agencies.

Our work also involves surveillance of these products after approval, involving inspections, assessing adverse-event reports, investigating these adverse-event reports, as well as investigating product failures. The legal framework that is necessary in order to do this work involves a working knowledge of the federal regulations, the various user fee agreements involving drugs and devices, the FD&C Act, as well as knowing about the guidances. We are also involved, of course, in writing guidances, which are FDA guidances, but also include international guidances, working with regulatory agencies from other countries.

The regulatory process itself involves decision making. This is based primarily on scientific data showing safety, efficacy, and purity. The decision-making process involves, among other things, internal review, presentations to this committee, meeting with manufacturers. Meeting with manufacturers is throughout the development of the product, even prior to the submission of an IND. We have pre-IND and pre-IDE meetings, depending on whether this is a biologic or a device, and IND meetings, pre-BLA -- in other words, meeting prior to the license application -- and then during the license application review, different meetings with the company and what is listed here as the important late-cycle meeting.

The other tasks that the division is involved in besides review of these applications and research involve writing policy and guidance documents, harmonization with other regulatory agencies, writing guidance with the International Committee for Harmonization, and having meetings with other regulatory agencies, such as the EMA organization, and also meeting with the Canadian Health Authority on a regular basis. We also have regular liaison meetings with other government agencies, including the NIH and the CDC. We meet regularly with different groups that represent industry, such as PPTA and AABB, and with international bodies such as WHO and ICH, and with patient groups such as the NHF, National Hemophilic Foundation, and the IDF, the Immune Deficiency Foundation. Recently we have instituted meetings with patient focus groups. Last year there was a focus group meeting with patients regarding patients with bleeding disorders.

In addition to all of this, we are involved with communications with the outside world involving posting on websites, writing letters, such as “Dear Doctor” letters. We are very active in having workshops to deal with issues that relate to the regulation. We have been involved with working on citizen petitions as well.

Just briefly, going over the types of research that are being performed in the division -- and, as you will see, it is closely related to the products that we regulate:

• We have research topics in coagulation. This involves standard and assay development.

• Also looking at the immunogenicity of proteins. This is a very important field.

• We also have research involved in recombinant technology, looking at polymorphisms, particularly SNPs.

• Looking at protein structure and function, and important research is involved in looking at aggregates and developing techniques to detect these aggregates early, as they are associated with adverse events.

• We also have research involved in looking at blood-borne viruses, such as hepatitis C.

• Oxygen-carrying compounds.

• Platelet function and storage, prevention of contamination of blood products.

• Also research involving red blood cells -- and quite an important study recently published comparing old cells versus new cells.

• We have several projects related to counterterrorism.

Just a very quick look at the workload. This is a very crude look. If you look at the top row, the PIs, or principal investigators, what is listed here is that they do 50 percent review and 50 percent research. This depends on, at a particular time, what is coming across their desks. If they have a license application coming across their desk and they are the chairpersons of that license, I guarantee you they will do more than 50 percent review. The same applies to staff fellows. We have a staff fellow program. They are also expected to do at least 50 percent review. The ORISE fellows are contract fellows. They are not cleared to do review, so they are spending 100 percent of their time doing research. Biologists who are assistants for the PIs are also participating very heavily in review.

I thank you for your attention.

DR. JACKSON: Thank you very much, Dr. Golding.

Any questions for Dr. Golding?

(No response)

It has only been 50 minutes since we started. Is it okay if we just move ahead with the next talk instead of taking a break at this point? If that's the case, Dr. Vostal will be speaking and giving an overview of the Laboratory of Cellular Hematology.

Agenda Item: Overview of the Laboratory of Cellular Hematology

DR. VOSTAL: Good afternoon. Thank you very much for your patience.

What I'm going to do in the next few minutes is to give you a summary of the site visit that happened earlier this year. The site visit actually took about four hours to complete. What I am going to present to you today is going to be the Reader’s Digest version of what went on.

You already heard about our laboratory, the Laboratory of Cellular Hematology. We are nestled here, one of four laboratories in the Division of Hematology. We are also in the Office of Blood.

I’m going to start off by talking a little bit about the regulatory responsibilities that we have in our laboratory. The products that we review are the transfusion products. These are red cells. They come in a number of different types -- whole blood-derived, apheresis-derived, 24-hour hold, pathogen-reduced. Similarly for platelets. There are all different types of platelets. For platelets, we also look at uniquely stored products, such as frozen platelets, platelet substitutes, or platelet-like hemostasis agents. We also do a little bit of plasma for whole blood and apheresis and 24-hour hold products.

All these transfusion products are developed through the use of devices. We review devices as well. These include the blood collection and storage bags, the apheresis instruments for all the different cell and transfusion product types, leukocyte-reduction filters, pathogen-reduction processes, bacterial detection, both culture-based and rapid-based, leukocyte-counting devices, platelet antigen testing kits, and blood warmers. So a lot of different and variable devices.

Some of these devices also come with drugs to support the anticoagulation of those products, such as anticoagulants, additive solutions for red cells and platelet storage, pathogen-reduction chemicals, and rejuvenation solutions for red cells.

So quite a diverse portfolio of review responsibility comes through our lab.

The scope of our regulatory review is to look at the preclinical evaluations of products and devices for safety and efficacy, blood compatibility for devices, product and material toxicology, review the software, clinical trial design and evaluation, and also look into product failures that are reported to the FDA and the adverse events that are reported. So we do have a pretty significant regulatory workload.

This is an example of what we went through in the year 2013. For the drug-based supplements and annual reports, we saw 91 of those. For the devices, we saw 45. For the clinical trial-associated amendments and original applications, we saw 86 of those. We had 15 meetings with sponsors. These are pre-submission meetings to explain to the sponsors what will be necessary to get their product on the market.

This regulatory load is combined with very tight timelines for review. I just want to give an example of some of these. For example, for the clinical trial supplements, we only have 15 days to review that, for the preliminary review for the devices we have 45 days, and for the drugs we have 90 days. So this type of timeline with this type of workload does amount to a significant amount of effort for the people who are in our laboratory.

Now I’m going to talk more about the research that we do. We have three principal investigators in the lab. It’s myself, Dr. Simak, and Dr. Atreya. The principal investigators are supported by research staff. I’m not going to go through every name on this, but I want to point out that there are staff scientists who are permanent employees and stay with the principal investigator, and there are ORISE fellows that are on a contract basis, and they do rotate through the labs on a temporary commitment.

The research in our laboratory is aimed to support the regulatory mission of CBER. It develops expertise in the transfusion products regulated by the LCH reviewers. The outcome of this is that the reviewers have a very deep understanding of the biochemistry and cell biology of the cellular products that they regulate. Our research investigates adverse events associated with regulated transfusion products, and the research evaluates new methods and new materials for storage of transfusion products.

We also investigate some of the major public health issues associated with transfusion products. As an example, here are reports to the FDA for the years 2009 to 2013. These are reports of fatalities associated with transfusions. They are organized by the type of adverse event. You can see that TRALI, transfusion-related acute lung injury, takes the lion’s share of these reports. We actually do research on TRALI. The TRALI gets reported to the FDA. The mortalities are coming from red cells and also from apheresis platelets.

The other major percentage of reports reported to the FDA for mortalities comes from microbial infections. These are strongly linked to platelet products because of their storage at room temperature.

Here are the research projects in the Laboratory of Cellular Hematology. My project is on safety and efficacy of platelet and red cell transfusion products. Dr. Simak’s project is on blood and vascular toxicity of engineered nanomaterials and biological microparticles in blood and their biomarker applications. Dr. Atreya’s project is on novel approaches to microbial reduction in *ex vivo* stored platelets and the study of blood cells in storage towards enhancing product shelf-life.

I’m going to just give you a brief summary of the projects that I do under the umbrella of safety and efficacy. We are looking into platelet transfusion-related acute lung injury, or platelet TRALI. We have a model to look at the events that happen after platelet transfusion. We also have a red cell project that deals with the evaluation of a potential new red cell transfusion product, which would be a stem cell-derived red cell.

To get into TRALI, the definition of TRALI is a transfusion-related acute lung injury which is the onset of acute respiratory distress, with new or worsening bilateral infiltrates on chest X-ray, without the evidence of a circulatory overload, occurring within six hours of transfusion. It is generally agreed upon that this mechanism involves two steps, referred to as two hits to the patient. The first hit is a general inflammatory response that is coming from either sepsis or previous chemotherapy or surgery. It sets up the patient by priming their neutrophils and having the neutrophils retreat back into the lung. The second hit is the transfusion that activates the prime pulmonary neutrophils. The mechanism of this activation is either through donor antibodies -- that is, donor antibodies through the recipient’s neutrophils or leukocytes -- or by biologic mediators that are released by the stored cells. Some of these have been identified as lipids, peptides, and microparticles.

We are also focusing on a relatively new mechanism. These are activated and damaged platelets -- so non-antibody-mediated, but also sufficient to activate the neutrophils and cause the acute lung injury.

The antibody-mediated TRALI occurrence has been reduced by selecting donors with lower antibody frequency. This would be selection for male donors. Therefore, the non-antibody-mediated TRALI is becoming a more prominent mechanism.

Some of the research that we do is also product-related. As an example of this, we have a product that was recently approved. These are pathogen-reduced platelets by Cerus, called the INTERCEPT Blood System. In the warnings and cautions in the labeling of this product, the manufacturer puts out the statement that the INTERCEPT-processed platelets may cause the following adverse reaction: acute respiratory distress syndrome. That’s because in a clinical trial with these products 1.6 percent of patients who received INTERCEPT-processed platelets came down with some acute respiratory distress, as compared to zero patients in the control arm. So there may be some kind of a connection between treated platelets and acute lung injury.

We have been wondering about this type of connection. We thought one way to approach this would be to set up a transfusion model to see if we could reproduce

TRALI in an animal model. Our animal model involves immunodeficient mice that can accept human cells. The first hit that we give them is treatment with LPS. That is a bacterial extract, so it sets up their immune system. Then the second hit is by platelets that have been -- these are human platelets -- collected by apheresis and exposed to UVB light. After the second infusion and a certain amount of time, we follow the distribution of the platelets in different organs. We look at tissue histology. We can track the platelets in the circulation of those animals.

Here is an example of a data set that we produce in this type of a model. This is immunofluorescence of lung tissue from an animal that has been infused with PBS, or salt solution, control human platelets, or UVB-treated human platelets. This is an animal that has been treated with LPS, either LPS and control platelets or LPS and UVB platelets. They may be a little difficult to see, but the human platelets show up as green fluorescence in this tissue. You can see that, if the animal received the two hits, the LPS and the UVB platelets, there is accumulation of human platelets in the tissue. Up here is a summary of a number of experiments. You can see that the two hits to the animal do increase significantly the number of platelets that end up in the lung.

When the platelets end up in the lungs, does that make any problems for the animals? The answer is yes. If you look at the lung histology, this would be normal histology of an animal infused only with saline, here is an animal infused with LPS, and here is the double-hit animal, with LPS and UVB platelets. You can see, when you compare it to the normal animal, that there is significant loss of pulmonary structure.

The hallmark of acute lung injury is protein in the alveolar fluid and leukocytes in the alveolar fluid. Again, if you look at the two-hit animal, you see an increase in protein, an increase in leukocytes under those conditions.

We have done a number of studies and published four papers to explain this model. This cartoon summarizes what we have learned. We learned that if you expose a platelet to UV light -- and this is UVB in particular -- it activates the platelets through protein kinase C. It causes expression of P-selectin on the outside of the platelet. It also changes the conformation of the fibrinogen receptor, so now the platelet can bind fibrinogen. If you take these platelets and infuse them into an animal that has been treated with LPS, they accumulate in the lung. That accumulation causes release of a cytokine called MIP-2. It’s this cytokine that activates the effector cells, neutrophils, and macrophages that cause the acute lung damage to the alveoli and cause protein leak and infusion of leukocytes.

We think under these conditions that this scheme actually suggests some potential for therapeutic intervention. We think that if we look at receptor blocker for MIP-2, or the human equivalent, which is IL-8, there may be some potential to reduce acute lung injury through this.

The other thing we learned from this model is that it’s not very sensitive. Human platelets actually don't bind to mouse pulmonary endothelial cells very well. The mouse platelets actually bind more efficiently and turn out to be a bridge between the endothelial cells and the human platelets.

We have looked at other rodent animal models that may have a better efficiency of binding human platelets, and we came up with an immunodeficient rat. We repeated this experiment in Rag2-negative rats. This is looking at pulmonary tissue from an animal that received LPS, LPS and control human platelets or LPS and UVB human platelets. You can see a comparison between the tissue from a mouse injected with human platelets, UVB-treated human platelets, and a rat treated with UVB human platelets. This distribution is a lot more uniform that you see in the mouse, so we think that the rat will turn out to be a more sensitive model.

For the TRALI model, our future plans are to compare the rat and the mouse, especially in terms of sensitivity, to explore the therapeutic approaches to reduce or prevent platelet TRALI. We think that looking at cytokine receptor blockage may be one way of doing that. We also think that we would like to investigate the role of platelet microparticles in this animal model.

So that brings me to our red cell project. What we are studying here -- we are anticipating the arrival of a new transfusion product, which would be a stem cell-derived, *ex vivo*-generated red cell. The technology is already existing to do this. It has been done in the test tube. I think it is just a matter of being able to scale up to process.

Stem red cells could improve on the current donor-derived red cells with respect to the supply issue, with respect to transfusion-transmitted disease, and also in minimizing the storage lesion. Our objective is to develop methods and a database to evaluate the stem red cells through comparison to conventional donor-derived red cells. The tests we are going to focus on are *in vitro* tests, such as oxygen binding, ATP generation, and cell fragility. We also would like to develop some functional tests -- for example, an animal model oxygen delivery by those cells.

In order to be able to develop some of these animal models and *in vitro* tests, we had to start off making our own stem cells in the laboratory. We have chosen cord blood stem cells as our starting material. You can see right here the pellet. They are totally white. If you put them in the right cytokine environment, in a matter of 18 days they actually mature into what looks like a pretty good red cell. Here is a comparison between conventional red cells and stem cell red cells. It is difficult to distinguish between the two just by looking at them in a test tube.

We have taken these cells and injected them into our animal transfusion model. We have done a comparison between the control red cells and the stem cell-derived red cells. You can see that the clearance curves are very close to each other, suggesting that the cells are recognized similarly by the animal.

If you look at these cells under the microscope, these are at day 0, the large nucleated stem cells that we start with. By day 18, you can see significant changes in these cells. Some of these cells have managed to get rid of their nuclei and they are starting to resemble normal-looking red cells. But it’s not all the cells. Our success in enucleation is about 50 or 60 percent. That’s about what everybody else has been reporting in the literature.

Just to summarize where we stand with this project, we have been able to achieve about 1000-fold proliferation. Our success rate in enucleation is about 50 to 60 percent. Our capacity is to produce about 3×10^9 cells per each culture that we do.

Looking at testing these cells, the stem red cells are comparable to conventional red blood cells in the *in vitro* biochemical tests, such as ATP levels and glucose metabolism, and also in the *in vivo* survival in an animal model using the SCID mouse.

There are significant differences that we have noted. Stem cells are different from conventional red cells in osmotic fragility. They are less fragile. They have a lot tighter oxygen binding. That's probably because they have a lot more fetal hemoglobin present. In our early tests of oxygen delivery in an animal model, there is a decrease in delivery in anemic exercise stress test.

For our future plans, we would like to improve the enucleation rate and our culture capacity so we can have more cells to test. We also would like to optimize the animal model of oxygen delivery by red cell transfusion.

So that finishes the summary of my projects. Now I'm going to talk about Dr. Simak's research.

Dr. Simak's projects deal with the *in vitro* evaluation of the effects of nanomaterials on blood cells and endothelial cells, and also the cell membrane and protein microparticles in blood and blood products, and their applications as biomarkers or their potential to cause vascular injury.

In these engineered nanomaterials, Dr. Simak has tested them against blood platelets and vascular endothelial cells.

This is a report that came from Dr. Simak's lab. They have taken carbon nanotubes, which are a relatively common nanomaterial, and they have exposed it to human platelets. What they discovered is that these nanotubes can actually penetrate the plasma membrane very easily, but when the nanotubes penetrate the membrane, the membrane is capable of sealing around carbon nanotubes. Once they get inside the organelle membrane, the story is a little different. The carbon nanotubes can still penetrate inside the vesicles, but this time the seal is not perfect, and there is a calcium leak around the nanotubes. This calcium leak depletes the internal calcium stores and sends a signal to start an influx of calcium, causing a general platelet activation. So exposure of platelets to carbon nanotubes actually leads to a very strong signal for platelet activation.

Another report that Dr. Simak's group has been able to publish is with carboxylated carbon nanotubes in endothelial cells. This time around, they are exposing these carboxylated nanotubes to endothelial cells. These are labeled here with green fluorescent protein. In this slide you can see that the green is the cells plus the fluorescent-labeled carbon nanotubes. You can see how those carbon nanotubes ended up inside the cell, sort of like this cartoon points out.

There are so many nanotubes that go inside the cell that the cell actually goes through apoptosis. An interesting discovery that Dr. Simak made was that if you incubate that with a low level of bafilomycin, the cell actually is capable then of extruding or releasing these carbon nanotubes in extracellular vesicles, and it actually improves the survival of the cells, as you can see over here, between control and control and bafilomycin-treated cells. So an interesting potential treatment for nanotoxic material toxicity.

The future plans for this project:

• To investigate the effects of nanoparticles and nanoparticles exposed to plasma so they have an immunoglobulin protein corona -- to test this complex particle then on platelets and endothelial cells.

• To optimize and validate a panel of *in vitro* assays so they can be used to evaluate the platelets and cultured cells when they become exposed to nanomaterials.

• Also to look at the effects of these nanomaterials on the plasma coagulation system.

The second project that Dr. Simak's lab is doing is the characterization of platelet vesiculome, looking at the different types of platelet microparticles that are released by these cells. Platelets release microparticles during activation with agonists or during storage or during certain types of preservation, such as freezing in DMSO. Dr. Simak's laboratory has worked out a number of methods to track and quantitate the different types of microparticles.

This is an interesting system. They are able to label the outside of the platelets with a specific dye and also label the inside membranes of vesicles with a separate dye. Then, stimulating these cells, you can follow particles that are released either from the plasma membrane or from the intracellular organelles. This can be tracked very nicely through confocal microscopy.

This is another cartoon and a little bit of data on what happens to platelets and their microvesicles. When the cells release plasma-based microvesicles, you can see the blebbing on the outside of the platelets. A second population of microvesicles is also stored on the inside. You can see this through transmission electron microscopy and scanning electron microscopy.

Their focus is also on very small microparticles. This new method will allow them to study that. This is atomic force microscopy analysis of microparticles that come down with 100,000 times *g* -- very small microparticles and a nice method to look at the characteristics of those microparticles released under different conditions from platelets.

They have applied some of these methods to look at platelets that have been stored in DMSO. DMSO is actually quite a good cryoprotectant when it is used to store stem cells. You can freeze stem cells in DMSO and thaw them, and they thaw and are actually quite functional. The same is not true for platelets. When they are frozen in 6 percent DMSO, which is the standard method, there still is substantial damage to the cells. This is associated with a membrane transition, with vesiculation, with loss of intracellular integrity, loss of platelet reactivity, and an establishment of a procoagulant phenotype. Some of these aspects may actually be good in a product, if you want to use it as a procoagulant to stop acute bleeding. But it’s difficult to compare these types of platelets with conventional platelets.

Here is a comparison of conventional platelets and DMSO-frozen platelets. You can see that conventional platelets, liquid-stored, are nice and smooth by scanning electron microscopy. An obvious difference is the damage caused by the freeze-thaw process and activation.

They have also looked at microparticles released by DMSO freezing through flow cytometry. This is looking at microparticles released from platelets, staining with annexin V to detect phosphatidylserine-expressing microparticles. If you compare just the number of microparticles released between the two cell products, you can see that DMSO-frozen platelets have a lot higher release of microparticles. These are positive for annexin V binding, so likely a procoagulant mechanism for the effects of those platelets.

To summarize and talk about the future plans, the future plan is:

• To develop an assay for characterization of membrane and protein submicron particles in biologics -- a general application to all sorts of micropraticles.

• To characterize the DMSO-frozen platelets and develop a panel of *in vitro* assays for QC and lot release.

• To investigate the membrane transition changes during DMSO-freezing and try to develop new methods of cryopreservation to achieve a fully functional platelet.

So that summarizes Dr. Simak's research projects. Now I'm going to move on to research in Dr. Atreya's lab.

There are two projects here. One is focused at looking at developing antimicrobial peptides that could be used to eliminate or reduce bacteria in stored platelets. The other project is to understand the changes that take place during platelet and red cell storage, and to use those changes to try to improve storage so you could decrease the storage lesion.

The first project is focused on reduction of bacteria. This is done by these peptides. The peptides are actually released. Some of the peptides are released by the platelets themselves and some of the peptides are synthesized. They have set out looking at the activity of these peptides against bacteria. They are looking for broad-spectrum activity.

They did find some of that activity, and they have proceeded to look at the preclinical safety analysis of these peptides. They have done an *in vivo* safety analysis in SCID mice, the transfusion model. They have looked at immunogenicity of the peptides in rabbits. They have also looked at the *in vitro* analysis of platelets that have been exposed to these peptides.

These peptides are relatively small. They are about six to ten amino acids. Some of them are found in nature and some are synthesized. Dr. Atreya's lab has designed a number of peptides, either singly or in combination with each other, and have tested these against common Gram-positive and Gram-negative bacteria.

Here is a slide of representative data that they have. We are looking at activity against *Staph. aureus*. The control is 4 logs of growth. Then you can go down the line and see the different types of peptides. You can see that some of them are active on their own, but most effective is the combination of these peptides. This is a very similar profile to what you see against other bacteria. So a certain combination of these peptides could be very effective at reducing the bacterial contamination in platelet products.

Then Dr. Atreya's lab went on to test the effects of these peptides on platelets. They incubated the peptides with human platelets for two hours and then infused them into the SCID transfusion model. You can see that the clearance curves of those platelets, whether they have been stored with saline or a peptide, are very similar, suggesting that the peptides do not change or activate the human platelets.

They have then taken the same peptides and injected them into rabbits to measure their immunogenicity. Results of those studies were negative for a generation of antibodies.

Future directions for this peptide work: They want to look at the toxicity evaluation of the selected peptides in small animal models, to develop a proof-of-concept that these peptides can be used to reduce bacterial contamination in stored platelets. They especially want to look at clinical bacterial strains, which may be a more difficult problem, but which are the ones that are frequently encountered as contaminants in platelet transfusion. Again, they want to look at screening and evaluation of other peptides to see if they could even increase their bactericidal activity.

The second project in Dr. Atreya’s lab is to try to understand the storage lesions that happens during storage of red cells and platelets. It’s pretty well known that stored cells, even under the best conditions that we have, go through some biochemical, morphological, and immunological changes. There is a great deal of concern that once these products are transfused, there could be negative clinical consequences, such as acute lung injury, multi-organ failure, or mortality.

Dr. Atreya’s lab has decided to take a different approach, and using a state-of-the-art technology, they are investigating the role of microRNAs in the storage lesion of red cells and platelets. MicroRNAs are established as the key cellular regulator of genes in a eukaryotic cell. This is actually even more important in cells that are enucleated, such as red cells and platelets, because microRNA is the only major regulatory nucleic acid that is identified that can regulate cellular processes. The goal is to understand the storage lesion process through microRNA regulation. Hopefully this will provide some clues for how things could be changed to optimize storage conditions.

Dr. Atreya's lab started out by looking at different phases of the storage lesion, including red cell apoptosis, red cell ATP generation or loss, and the mean cell volume during the course of storage. Each one of these changes is associated with a number of different microRNAs. They identified 92 microRNAs that were shared by all three. Out of these 92, they identified two that expressed differently over time, suggesting that there may be some kind of a dynamic effect going on. So they focused on those two that were changing during the course of storage.

They were able to take one of them, 196, and express it in an erythroblast cell line. Once this microRNA was expressed, it actually increased the level of ATP in these cells and decreased their annexin V binding assay, which suggests that they have a reduced apoptosis rate going on -- so an experiment that does suggest that this microRNA may have some potential role in regulating what happens to a cell in storage.

In summary, the analysis showed that two microRNAs, 196 and 1269, change in expression levels. This is correlated with different parameters of red cell storage lesion. Their differential expression was confirmed by RT-qPCR analysis. Looking at the data that is published in the literature, they identified potential targets or regulators of biochemical reactions. Overexpression in one of those microRNAs in an erythroblast cell line seemed to be protective against cell death and ATP loss.

These are encouraging results, but they still need to be tested in actual human red cells.

In support of this concept that microRNAs can protect red cells from the storage lesion, other investigators have published that different microRNAs can protect erythrocytes against oxidation stress. So it appears that this concept is solid, and I think it deserves further investigation.

The future plan is to look at the role of microRNAs in the mechanisms of storage lesions. The objective here is to improve the quality of the red cells and platelets after storage.

That pretty much summarizes the research going on in our lab. I would be happy to answer any questions.

DR. JACKSON: Are there questions for Dr. Vostal or any of the speakers from the committee members?

(No response)

It’s still early. Is it okay if we do the open public hearing? I think we only have one person signed up at this point.

Agenda Item: Open Public Hearing

Before we start, I need to make this announcement.

Welcome to the open public hearing session. Please state your name and your affiliation, if relevant to this meeting. Both the Food and Drug Administration, FDA, and the public believe in a transparent process for information gathering in decision making. To ensure such transparency at the open public hearing session of the advisory committee meetings, FDA believes that it is important to understand the context of an individual’s presentation. For this reason, FDA encourages you, the open public hearing speaker, as you begin, to state if you have any financial interests relevant to this meeting, such as a financial relationship with any company or group that may be affected by the topic of this meeting. If you do not have any such interests, also FDA encourages you to state that for the record. If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking and you may still give your comments.

I believe we a Mr. Peter Sprigg from the Family Research Council here.

MR. SPRIGG: My name is Peter Sprigg. I’m a senior fellow for policy studies at the Family Research Council in Washington.

I have no financial interests.

I wanted to address the issue of blood donor deferral of men who have sex with men. You will have to forgive me. I just came back in the room. I’m trying to bring up my remarks on my laptop, and it’s taking me a little bit of time. I had these well-prepared remarks, which I may just have to give extemporaneously.

I want to affirm the members of the committee for your faithful work on this issue. It was my privilege to address you at your previous meeting on December 2. I was gratified, following my remarks, during the discussion time, that a number of people on the committee expressed some of the same concerns that I shared. In fact, these have also been recounted in the summary minutes of the meeting. For example, a concern was expressed that MSM might misunderstand a one-year deferral as a general message that MSM in general may safely donate blood. Many members of the committee commented that the decision for a policy change related to deferral of MSM as blood donors needed to be science-based. Certain members said that an abundance of data to support a change in policy has not been demonstrated and that safety of a revised policy is uncertain. One member stated that the usual approach to blood safety is based on the precautionary principle, and a change in deferral policy should not be made if there is a potential for increased risk to the blood recipient. Certain members commented that blood donation is not considered a civil right, and many groups are excluded from blood donation because of various risk factors.

The odd thing is what did not happen at the December meeting, which is that this committee did not vote to recommend a change in the current blood donor deferral policy for men who have sex with men. In fact, no vote on such a recommendation was even taken. It is striking to me that even today you have heard explicitly from the FDA that they are not seeking the advice of their advisory committee on this particular topic.

What that suggests to me is that the fix is in and the decision has already been made, not because of scientific or medical reasons, but for political reasons, in order to appease a small but very vocal special-interest group. I think that this is inexcusable for an agency that is supposed to be dedicated to protecting the public health, and I would urge the FDA to halt the proposed change in policy and, instead, heed the concerns that were raised in December by members of this committee.

Thank you.

DR. JACKSON: Thank you.

Is there anyone else in the audience that has any comments on any of the topics this afternoon?

(No response)

Okay, I think this ends the public hearing session.

At this point, our public session is over. We are going to take a short break and come back for the executive session. Mr. Emery will clear the room of those non-members.

(Whereupon, at 4:00 p.m., the open session was concluded.)